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AUTOIMMUNITY IN MYASTHENIA GRAVIS: A FAMILY STUDY

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SUMMARY

The prevalence of autoantibodies to muscle, epithelial cells of calf thymus, thyroid, gastric parietal cells and antinuclear and rheumatoid factors has been studied in the sera of thirty-two patients with myasthenia gravis and their relatives.

Previous reports of an increased prevalence of autoantibodies in the sera of patients with myasthenia gravis have been confirmed and it has been shown that concurrent reactivity to muscle and thymus is closely correlated with the severity of the myasthenia and the presence of a thymoma, whereas no such correlation occurred with the other antibodies studied. None of the sera from relatives or spouses showed concurrent reactivity with thymus and muscle, and with the exception of one patient with pernicious anaemia, sera from patients with a variety of other diseases were also negative.

A slight increase in the prevalence of autoantibodies to thyroid and gastric components and antinuclear factor was found in first degree relatives of patients with myasthenia gravis; this could be accounted for by their aggregation in a few families.

INTRODUCTION

Autoantibodies to muscle were first demonstrated in the serum of patients with myasthenia gravis by Strauss *et al.* (1960) using complement fixation and confirmed by Beutner *et al.* (1962) using an indirect immunofluorescent technique. Since then a number of reports have appeared which indicate that these antibodies are present in sera from 25% to 50% of patients with myasthenia gravis (Feltkamp *et al.*, 1963; van der Geld *et al.*, 1963; Osserman & Weiner, 1965; van der Geld & Strauss, 1966). The antibody reacts with A-bands in skeletal and heart muscle but not with the motor end plate. Concurrent reactivity between striated muscle and epithelial cells of calf thymus has been demonstrated (van der Geld, Feltkamp & Oosterhuis, 1964; van der Geld & Strauss, 1966). Similar reactivity was noted in only one healthy subject and did not occur in patients with other disorders of muscle.

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The antibodies are found most commonly in patients with an associated thymoma and in those with severe or progressive disease. In addition, a high incidence of antinuclear factor (White & Marshall, 1962) and thyroid antibodies (van der Geld *et al.*, 1963) has been described in myasthenia gravis, suggesting that there is a widespread autoimmune disturbance in the disease.

Additional evidence in support of the possibility that an autoimmune disturbance might be a factor in the pathogenesis of myasthenia gravis might be obtained by examining its association both clinically and serologically with other putative autoimmune diseases. Since autoimmune diseases frequently run in families, a similar study of the relatives of patients with myasthenia gravis would be of interest.

It is the purpose of the present report to describe the distribution of autoantibodies in the sera from thirty-two patients with myasthenia gravis and their relatives as well as in the sera of patients with a variety of other disorders. The clinical aspects of the study will be reported in detail elsewhere (Kerr & Wright, in preparation).

MATERIALS AND METHODS

Patients studied

Myasthenia gravis probands and relatives. Thirty-two patients with myasthenia gravis were interviewed and a full personal and family history obtained. The accessible relatives were visited and specimens of blood taken from all the propositi, 106 blood relatives and twenty spouses.

Thymoma probands and relatives. Four patients who had had a thymectomy for a thymoma but had no clinical evidence of myasthenia gravis and their relatives were contacted in the same way and specimens of blood taken; a total of ten relatives and three spouses.

Miscellaneous diseases and healthy controls. Additional specimens of serum were available from fifty patients with pernicious anaemia, fifty patients with rheumatoid arthritis, fifty patients with thyrotoxicosis who had been treated with radio-iodine, twenty patients with systemic lupus erythematosus and a large number of healthy controls who were matched for age and sex with the test groups.

Serological tests

All specimens of serum were given a code number and stored at $-20°C$ in several small aliquots until tested for the following autoantibodies:

(1) Antibodies to muscle and epithelial cells of calf thymus. These were detected by means of the indirect immunofluorescent technique. Sections of rat, human and calf muscle and calf thymus 5–8 μ thick were cut in a cryostat at $-20°C$ after the tissues had been snap-frozen in iso-pentane. Serum at an initial dilution of 1:10 was applied to the sections for 10 min and then washed in barbitone buffer at pH 7·2 for 60 min. A high-titre rabbit anti-human IgG globulin conjugated with fluorescein isothiocyanate (protein content 8 mg/ml, fluorescein–protein ratio of 4, 8 units of anti-IgG antibody per ml; Beutner, Holborow & Johnson, 1965), kindly provided by Dr E. H. Beutner, was diluted 1:40 and applied to the sections for 10 min in a humid atmosphere. After washing for 120 min the sections were

mounted in equal volumes of glycerol and buffer. They were examined within 24 hr by fluorescence microscopy with an HB 200 lamp as the source of ultraviolet light and BG 12 excitor filter.

(2) Antinuclear factors were detected in a similar manner using sections of muscle and rat liver.

(3) Antibodies to thyroid acinar cell cytoplasm (Holborow *et al.*, 1959) and gastric parietal cells (Taylor *et al.*, 1962) were also detected by the indirect immunofluorescent technique as described elsewhere (Wright & Truelove, 1966).

(4) Antibodies to thyroglobulin were detected by the tanned red-cell test using preserved cells (Fulthorpe *et al.*, 1961) as supplied by Wellcome Research Laboratories, Beckenham, Kent.

(5) Sera were examined for rheumatoid factor by the Hyland latex test (Baxter Laboratories, High Wycombe, Buckinghamshire).

RESULTS

In Table 1 the prevalence of autoantibodies in the thirty-two patients with myasthenia gravis is compared with that in a healthy control group matched for age and sex.

TABLE 1. Autoantibodies in thirty-two patients with myasthenia gravis and thirty-two controls matched for age and sex

Antibody	Thymus epithelial cell	Muscle	Thyroglobulin		Thyroid cytoplasmic	Antinuclear factor		Gastric parietal cell
			Pos. $<1/40$	Pos. $\geqslant 1/40$		+	++	
Myasthenia gravis	8	6	3	5	9	6	4	6
Matched controls	Nil	Nil	3	1	5	1	1	2

Six of the patients with myasthenia gravis had antibodies to skeletal and heart muscle and epithelial cells of calf thymus. Two further patients showed reactivity with thymus epithelial cells but not to muscle. The reactivity was retained at a serum dilution of 1:60 or greater. None of the healthy controls were positive for muscle or thymus. The patients with myasthenia gravis were more likely to have antibodies to thyroid and gastric parietal cells and antinuclear factor than the control group.

In Table 2 it can be seen that the antibodies to muscle and thymus are more likely to be present in patients with severe disease but no such correlation exists with the remaining antibodies studied. Of the six patients who showed concurrent reactivity to muscle and thymus five had thymomas and the only other patient who had a thymoma associated with myasthenia gravis consistently showed a strong reaction to thymus but not to muscle. Two of the four patients with thymomas but no clinical evidence of myasthenia gravis showed concurrent reactivity with muscle and thymus.

In Table 3 the prevalence of autoantibodies in the sera from the spouses and first degree relatives of patients with myasthenia gravis is compared with that in healthy control subjects matched for age and sex. It can be seen that none of the relatives or the healthy controls

showed concurrent reactivity with muscle and thymus epithelial cells. The father of one of the propositi showed reactivity with thymus at a serum dilution of 1:60 but not with muscle.

TABLE 2. Autoantibodies in thirty-two patients with myasthenia gravis in relation to clinical severity

Clinical grading*	No. of patients	Thymus epithelial cell	Muscle	Thyroglobulin		Thyroid cytoplasmic	Antinuclear factor	Gastric parietal cell
				Pos. $<1/40$	Pos. $\geqslant 1/40$			
0	4	Nil	Nil	1	Nil	1	1	Nil
I	6	Nil	Nil	1	1	2	2	3
II	19	5	3	1	4	5	7	3
III	3	3	3	Nil	Nil	1	Nil	Nil

* 0, In remission; I, ocular myasthenia gravis; II, moderate generalized myasthenia gravis; III, severe generalized myasthenia gravis.

The relatives of patients with myasthenia gravis were more likely to have antibodies to thyroid and antinuclear factor than the matched controls but the differences were not significant.

TABLE 3. Autoantibodies in the sera of relatives of patients with myasthenia gravis and healthy controls matched for age and sex

Antibody		Myasthenia gravis relatives				Matched controls			
		Neg.	+	++	Total	Neg.	+	++	Total
Thymus*	Spouses	20	—	—	20	20	—	—	20
	First degree relatives ♀	46	1	—	47	47	—	—	47
	First degree relatives ♂	37	1	1	39	39	—	—	39
Muscle*	Spouses	20	—	—	20	20	—	—	20
	First degree relatives ♀	47	—	—	47	47	—	—	47
	First degree relatives ♂	39	—	—	39	39	—	—	39
Anti-nuclear* factor	Spouses	18	2	—	20	19	1	—	20
	First degree relatives ♀	42	3	2	47	44	2	1	47
	First degree relatives ♂	34	2	3	39	38	1	—	39
Thyroid cytoplasmic	Spouses	14	1	5	20	19	—	1	20
	First degree relatives ♀	34	6	7	47	42	3	2	47
	First degree relatives ♂	32	4	3	39	36	2	1	39
Thyroglobulin†	Spouses	12	7	1	20	16	4	—	20
	First degree relatives ♀	31	10	6	47	39	8	—	47
	First degree relatives ♂	29	4	6	39	34	5	—	39
Gastric parietal cell	Spouses	19	1	—	20	20	—	—	20
	First degree relatives ♀	44	3	—	47	46	—	1	47
	First degree relatives ♂	37	2	—	39	39	—	—	39

Thyroid and gastric staining graded by intensity.

* Positive + titre, $<1/60$; Positive ++ titre, $\geqslant 1/60$.

† Positive + titre, $<1/40$; Positive ++ titre, $\geqslant 1/40$.

When the individual families were examined it was found that the difference could be accounted for by the aggregation of most of the strongly positive thyroid antibodies and antinuclear factor in a few families. This is illustrated in Fig. 1. It is of interest that both

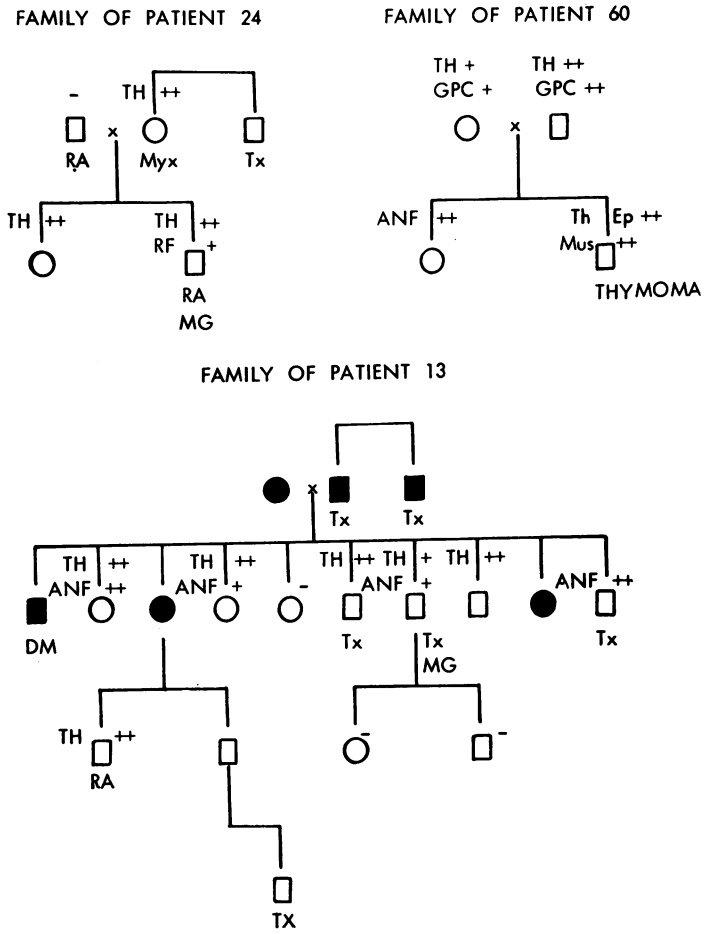

FIG. 1. Family trees of two patients with myasthenia gravis (24 and 13) and one patient with a thymoma (60) to indicate the aggregation of autoantibodies in the relatives.

Code, Circles, male; squares, female; shaded, deceased.

TX, thyrotoxicosis; MG, myasthenia gravis; DM, diabetes mellitus; RA, rheumatoid arthritis; Myx, myxoedema.

Antibodies: TH, thyroid, GPC, gastric parietal-cell; ANF, antinuclear factor; RF, rheumatoid factor; Th Ep, thymus epithelial cells; Mus, muscle.

organ specific autoantibodies and non-organ specific autoantibodies occurred in some families.

There was no increase in the prevalence of rheumatoid factor, the latex test being positive in only one patient with myasthenia gravis, four first degree relatives and one spouse. The

results in the patients with other diseases are shown in Table 4. Only one patient, an 85-year-old woman with pernicious anaemia and a strong family history of pernicious anaemia, showed high-titre concurrent reactivity to muscle and thymus. Weak reactions to thymus but not to muscle were found with some of the sera in each group.

TABLE 4. Autoantibodies to muscle and epithelial cells of calf thymus

	Thymus epithelial cells				Muscle			
	Neg.	Pos. $<1/60$	Pos. $\geqslant 1/60$	Total	Neg.	Pos. $<1/60$	Pos. $\geqslant 1/60$	Total
Thyrotoxicosis	48	2	Nil	50	50	Nil	Nil	50
Pernicious anaemia	47	2	1	50	49	Nil	1	50
Rheumatoid arthritis	49	1	Nil	50	50	Nil	Nil	50
Systemic lupus erythomatosus	20	Nil	Nil	20	20	Nil	Nil	20

DISCUSSION

The present study shows that antibodies to muscle and thymus are strongly correlated with the severity of the myasthenia gravis and the presence of a thymoma. The antibodies were also found in two out of four patients with a thymoma but no clinical evidence of myasthenia gravis. These results confirm the findings of van der Geld & Strauss (1966). One patient had antibodies in the serum 7 years after removal of a thymoma but had never shown clinical signs of myasthenia gravis. Another patient in whom antibodies to thymus and muscle had been found after removal of a thymoma subsequently developed myasthenia gravis without any change in the titre of antibodies.

The clinical findings raise the possibility that the stimulus to antibody formation arises in the cells in the thymus and that the antibodies so formed react with similar or identical antigens present in muscle. If an autoimmune disturbance is of importance in the pathogenesis of myasthenia gravis it might act on the thymus rather than on muscle or the motor end plate, the characteristic muscular weakness being produced in some other way which need not be immunological. Gordon, Hess & Frederick (1965) have discussed these possibilities but were unable to demonstrate significant *in vivo* binding of γ-globulin to muscle in patients with myasthenia gravis and therefore concluded that the antibodies were probably a secondary manifestation of the disease. On the other hand, examination of sera from a wide variety of patients with disorders of muscle have failed to demonstrate concurrent reactivity with muscle and thymus. It is of interest that *in vivo* binding of γ-globulin to thymus in patients with myasthenia gravis who did not have circulating antibodies to muscle or thymus has been demonstrated by van der Geld & Oosterhuis (1966).

The experimental work of Marshall & White (1961) supports the possibility that an autoimmune disturbance in myasthenia gravis might be initiated in the thymus. They produced germinal follicles in the thymus in guinea-pigs resembling those frequently seen in the thymus in patients with myasthenia gravis by injecting foreign antigen directly into the gland. Since germinal centres are sometimes found in the thymus in patients with

systemic lupus erythematosus, thyrotoxicosis and rheumatoid arthritis (Mackay & de Gail, 1963; Gunn, Michie & Irvine, 1964) and as enlargement of the thymus as determined by pneumomediastinography may be a feature in patients with pernicious anaemia and thyroid disease (Irvine & Sumerling, 1965; Irvine, Davies & Sumerling, 1965) we examined the sera of patients with these putative autoimmune diseases for antibodies to muscle and thymus. Only one patient—an elderly woman with pernicious anaemia—had antibodies to both muscle and thymus and none of the healthy controls or the relatives of patients with myasthenia gravis showed such reactivity. This confirms the high degree of specificity of these antibodies for patients with thymoma and myasthenia gravis and shows that similar genetic or environmental factors do not result in the development of these antibodies in unaffected relatives.

The findings of high-titre antibodies to muscle and thymus in a patient with pernicious anaemia is of special interest. She did not show clinical features of myasthenia gravis and conventional radiographs of her chest failed to demonstrate a thymoma. Four years previously she had had a hemiplegia from a cerebral thrombosis with some residual muscle wasting and weakness. Although van der Geld & Strauss (1966) and others have failed to find antibodies to muscle other than in myasthenia gravis it is possible that patients with certain diseases such as pernicious anaemia and myasthenia gravis are especially prone to develop organ specific circulating autoantibodies as a consequence of tissue damage. We have put this forward as one possible explanation for the finding that patients with gastritis associated with pernicious anaemia and idiopathic iron deficiency anaemia are more liable to develop gastric parietal cell antibodies than are patients with gastritis of similar severity associated with other diseases (Wright *et al.*, 1966).

The significance of the occasional reaction which we observed at low titre with thymus epithelial cells without concurrent staining of muscle is uncertain but is probably due to lack of sensitivity of the technique in our hands with the muscle preparation since van der Geld & Strauss (1966) have found that absorption with skeletal and heart muscle abolishes staining of the thymus and this is our own experience. Although the cells in the thymus which show positive immunofluorescent staining with certain sera have been regarded as epithelial cells by most workers, it seems more likely that they are striated muscle cells which can be demonstrated in the thymus using conventional histological techniques (Henry, 1966; Strauss, Kemp & Douglas, 1966) but this is a question that clearly requires further investigation.

Antibodies to thyroid and gastric components and antinuclear factor occurred more frequently in patients with myasthenia gravis than in a group of matched controls, but the numbers were too small for statistical analysis. This is in agreement with the findings of other workers (White & Marshall, 1962; van der Geld *et al.*, 1963; Downes, Greenwood & Wray, 1966). The prevalence of thyroid antibodies was greater in the relatives of patients with myasthenia gravis than in matched controls and a slight increase in antinuclear factor was also noted. It is of interest that these autoantibodies were not randomly scattered amongst the relatives of the patients with myasthenia gravis, but were aggregated in a few families.

Autoantibodies were found in a surprisingly large number of the spouses (Table 3). The numbers were small and differences from controls were not statistically significant but this finding emphasizes the need to include examination of spouses when undertaking family

studies. A high prevalence of autoantibodies in spouses would suggest that environmental as well as genetic factors are responsible for the aggregation of autoantibodies in certain families. These findings are in keeping with clinical observations indicating that a number of putative autoimmune diseases are occasionally associated with myasthenia gravis (Simpson, 1964; Reaves, 1965; Downes *et al.*, 1966; Singer & Sahay, 1966) and confirm our clinical observations that such diseases may also occur in their relatives (Kerr & Wright, in preparation).

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REFERENCES

BEUTNER, E.H., HOLBOROW, E.J. & JOHNSON, G.D. (1965) A new fluorescent antibody method: mixed anti-globulin immunofluorescence or labelled antigen indirect immunofluorescence staining. *Nature (Lond.)*, **208**, 353.

BEUTNER, E.H., WITEBSKY, E., RICKEN, D. & ADLER, R.H. (1962) Studies on autoantibodies in myasthenia gravis. *J. Amer. med. Ass.* **182**, 46.

DOWNES, J.M., GREENWOOD, B.M. & WRAY, S.H. (1966) Auto-immune aspects of myasthenia gravis. *Quart. J. Med.* **35**, 85.

FELTKAMP, T.E.W., VAN DER GELD, H., KRUYFF, K. & OOSTERHUIS, H.J.G.H. (1963) Antinuclear factor in myasthenia gravis. *Lancet*, **i**, 667.

FULTHORPE, A.J., ROITT, I.M., DONIACH, D. & COUCHMAN, K.G. (1961) A stable sheep cell preparation for detecting thyroglobulin autoantibodies and its clinical applications. *J. clin. Path.* **14**, 654.

GORDON, S.V., HESS, J.W. & FREDERICK, R.J. (1965) Myasthenia gravis. Studies on *in vivo* binding of γ-globulin to muscle. *Arch. intern. Med.* **115**, 405.

GUNN, A., MICHIE, W. & IRVINE, W.J. (1964) The thymus in thyroid disease. *Lancet*, **ii**, 776.

HENRY, K. (1966) Mucin secretion and striated muscle in the human thymus. *Lancet*, **i**, 183.

HOLBOROW, E.J., BROWN, P.C., ROITT, I.M. & DONIACH, D. (1959) Cytoplasmic localization of 'Complement-fixing' auto-antigen in human thyroid epithelium. *Brit. J. exp. Path.* **40**, 583.

IRVINE, W.J., DAVIES, S.H. & SUMERLING, M.D. (1965) The immunopathy of thyroid disease. *Proc. 5th int. Thyroid Conf., Rome*, 1965, p. 773.

IRVINE, W.J. & SUMERLING, M.D. (1965) Radiological assessment of the thymus. *Lancet*, **i**, 996.

MACKAY, I.R. & DE GAIL, P. (1963) Thymic 'germinal centres' and plasma cells in systemic lupus erythematosus. *Lancet*, **ii**, 667.

MARSHALL, A.H.E. & WHITE, R.G. (1961) Experimental thymic lesions resembling those of myasthenia gravis. *Lancet*, **i**, 1030.

OSSERMAN, K.E. & WEINER, L.B. (1965) Studies in myasthenia gravis: immunofluorescent tagging of muscle striation with antibody from serums of 256 myasthenia patients. *Ann. N.Y. Acad. Sci.* **124**, 730.

REAVES, L.E. (1965) Altered immunologic mechanisms in diseases of unknown cause. *Geriatrics*, **20**, 707.

SIMPSON, J.A. (1964) Immunological disturbances in myasthenia gravis with a report of Hashimoto's disease developing after thymectomy. *J. Neurol. Neurosurg. Psychiat.* **27**, 485.

SINGER, W. & SAHAY, B.M. (1966) Myasthenia gravis, Hashimoto's thyroiditis and pernicious anaemia. *Brit. med. J.* **i**, 904.

STRAUSS, A.J.L., KEMP, P.G., JR. & DOUGLAS, S.D. (1966) Myasthenia gravis. *Lancet*, **i**, 772.

STRAUSS, A.J.L., SEEGAL, B.C., HSU, K.C., BURKHOLDER, P.M., NASTUK, W.L. & OSSERMAN, K.E. (1960) Immunofluorescence demonstration of a muscle binding, complement-fixing serum globulin fraction in myasthenia gravis. *Proc. Soc. exp. Biol. (N. Y.)*, **105,** 184.

TAYLOR, K.B., ROITT, I.M., DONIACH, D., COUCHMAN, K.G. & SHAPLAND, C. (1962) Auto-immune phenomena in pernicious anaemia: Gastric antibodies. *Brit. med. J.* **ii,** 1347.

VAN DER GELD, H., FELTKAMP, T.E.W., VAN LOGHEM, J.J., OOSTERHUIS, H.J.G.H. & BIEMOND, A. (1963) Multiple antibody production in myasthenia gravis. *Lancet*, **ii,** 373.

VAN DER GELD, H., FELTKAMP, T.E.W. & OOSTERHUIS, H.J.G.H. (1964) Reactivity of myasthenia gravis serum γ-globulin with skeletal muscle and thymus demonstrated by immunofluorescence. *Proc. Soc. exp. Biol. (N. Y.)*, **115,** 782.

VAN DER GELD, H. & OOSTERHUIS, H.J.G.H. (1966) Auto-antibodies and myasthenia gravis: Epithelial cells of the thymus. *Ann. N. Y. Acad. Sci.* **135,** 631.

VAN DER GELD, H.W.R. & STRAUSS, A.J.L. (1966) Myasthenia gravis. Immunological relationship between striated muscle and thymus. *Lancet*, **i,** 57.

WHITE, R.G. & MARSHALL, A.H.E. (1962) The autoimmune response in myasthenia gravis. *Lancet*, **ii,** 120.

WRIGHT, R. & TRUELOVE, S.C. (1966) Auto-immune reactions in ulcerative colitis. *Gut*, **7,** 32.

WRIGHT, R., WHITEHEAD, R., WANGEL, A.G., SALEM, S.N. & SCHILLER, K.F.R. (1966) Autoantibodies and microscopic appearance of gastric mucosa. *Lancet*, **i,** 618.