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# A membrane-associated autoantibody in a case of myasthenia gravis with chronic hepatitis

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SUMMARY

A new antibody reacting with a cell membrane-related antigen is described in a patient with myasthenia gravis and chronic liver disease. The antibody gave a striking peripheral immunofluorescence in all cells tested and did not react with smooth muscle fibres, being distinct from anti-actin and anti-myosin antibodies. The antigen was widely distributed in human and animal tissues.

The exact relationship of this antibody to the clinical condition is not obvious but its presence is further evidence of widespread disturbances of humoral immunity in myasthenia gravis.

## INTRODUCTION

A number of autoantibodies give rise to a peripheral immunofluorescence pattern on various cells in a limited number of organs. Well-known examples are the anti-actin antibodies (Gabbiani *et al.*, 1973) which stain smooth muscle fibres and in addition react with the actin fibrils in kidney, liver and thyroid cells. Peripheral thyroid cell staining (Biberfeld, Fagraeus & Lenkei, 1974) is also seen in Yersinia infection (Lidman *et al.*, 1974), mononucleosis (Sutton *et al.*, 1974) and other diseases in the absence of SMA (Horne *et al.*, 1975) or peripheral hepatocyte IFL. The antibody described in the present case was unusual in having a widespread tissue distribution and was found in the serum of a patient with longstanding myasthenia gravis.

## MATERIALS AND METHODS

*Sera.* Three specimens were studied. The first and second were obtained 6 and 10 weeks after the first admission and the third 6 months later. The three specimens were titrated together for comparison.

*Immunofluorescence (IFL).* The sandwich technique was employed throughout, using FITC conjugates of anti-human immunoglobulins G, A, M, anti-$\beta_{1c}$, anti-kappa and anti-lambda. Titres were established with increasing serum dilutions to end point. Surface IFL of viable lymphocytes was done on a suspension of rabbit lymphocytes prepared as previously described (Linthicum & Sell, 1974).

*Tissue substrates.* The serum was tested on unfixed cryostat sections of tissues from human, rat, ox and sheep. The following organs were examined: human thyrotoxic thyroid, stomach, cartilage, brain, pituitary gland, adrenal, pancreas, submandibular gland; rat liver, kidney, testis, nerve, stomach, duodenum, ileum and colon, cervix, bladder, brain, lung, eye, striated and cardiac muscle; calf thymus; ox skeletal, cardiac and Purkinje tissue; sheep orbital muscle. Stretched glycerinated teased muscle fibres were used to establish the exact type of striational IFL, as skeletal muscle antibodies were also present in this serum.

*Fixation experiments.* Sections of liver and kidney were fixed in acetone, ethanol, methanol, ether, 1% formaldehyde and 0·5% glutaraldehyde for 10 and 30 min prior to IFL.

*Absorption of antibody.* Thyroid membranes were made from fresh human glands obtained at operation as used in the radioligand test for thyroid-stimulating antibodies (Smith & Hall, 1974). Serum dilutions of 1:100 and 1:1000 were incubated with equal volumes of membrane suspension starting with 1 g equivalent wet weight/ml in doubling dilutions for 1 hr at 37°C then overnight at 4°C. The mixtures were spun at 26,000 *g* for 30 min and supernatant tested by IFL.

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## RESULTS

*Case history*

A.M., a 61-year-old housewife, was investigated for unexplained progressive weight loss from 87 to 47 kg over a period of 5 years. For 30 years she had symptoms of slowly increasing, fluctuating muscle weakness from myasthenia gravis (MG), affecting mainly the limbs and recently eye and head movements, with little dysphagia. In 1970 she lost 16 kg in 2 months; abdominal tumour was suspected but not confirmed. At the time of admission in February 1975 she was emaciated and showed generalized pigmentation and liver palms. There was dyspnoea at rest. Investigations confirmed the myasthenia and indicated chronic airways obstruction. She was a regular smoker and had several attacks of pneumonia in the past. Although she had no jaundice or hepatomegaly and no past history of acute hepatitis, chronic liver disease was suspected as a cause of the pigmentation and weight loss since liver function tests were abnormal: alkaline phosphatase 21 KA units, SGOT 209 i.u., SGPT 340 I.U., HBsAg persistently negative. Liver biopsy showed 'chronic nongranulomatous inflammatory cell infiltrate mainly within portal tracts, with adjacent piecemeal necrosis and slight fibrosis. Scattered small foci of active liver cell necrosis. Overall architecture preserved. Features are those of chronic aggressive hepatitis' (Dr W. Jones Williams). Adrenal function tests proved normal. Nine months later, although her physical condition had deteriorated further, LFTs were normal and a second liver biopsy in November 1975 showed 'chronic persistent hepatitis with certain features of chronic active hepatitis (rosettes)'. Throughout the 9 months of study, the patient's serum contained the membrane antibody to be described but its titre fell gradually from 2560 to 160. Striational muscle antibodies connected with the MG were also present, as well as a trace of ANA. At no time were smooth muscle or mitochondrial antibodies detected.

*Description of cell-membrane-associated antibody*

The antibody was polyclonal, of IgG class, and did not fix complement. It stained cell membranes in all tissues of the species tested. There was a polygonal pattern on liver which appeared 'beaded' in some areas (Fig. 1). Bile duct cells were outlined. Blood vessels were stained on their outer and luminal borders (Fig. 2) but smooth muscle layers and portal tracts were entirely negative. Human thyroid cells were stained at their periphery. The appearance on these cells and on hepatocytes resembled that obtained with some anti-actin SMA sera. On rat kidney the glomeruli were positive with outlining of the mesangial cells. Peripheral staining was observed on all the tubular cells especially in the proximal tubules of which the third part reacted most intensely. In the gut, both mucus-secreting and glandular cells stained around their edges (Fig. 3). This appearance was also seen in submandibular gland where the duct cells were more strongly outlined than the acinar cells. In rat lung the alveolar cells were stained.

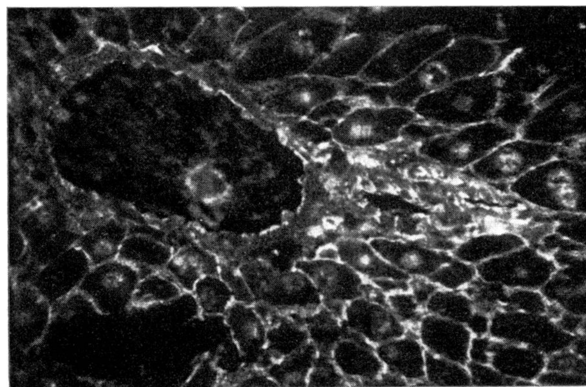

FIG. 1. Unfixed cryostat section of rat liver stained by indirect immunofluorescence with A.M. serum 1/10, and anti-IgG (Figs. 2–6 similarly prepared), showing beaded peripheral hepatocyte staining, and outlining of bile ductule cells. (Magnification ×225.)

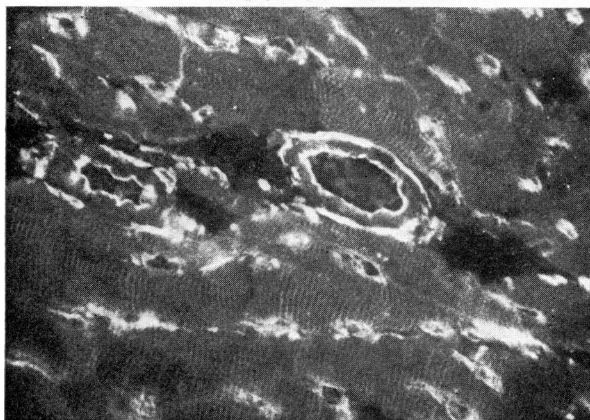

FIG. 2. Rat cardiac muscle and arteriole with pericellular staining of muscle fibres, vessel adventitia and endothelium. The vessel smooth muscle is completely negative. Striational staining of I bands is also present but of much lower titre. (Magnification ×225.)

FIG. 3. Rat duodenum with peripheral staining strongest at the luminal edge. (Magnification ×62·5.)

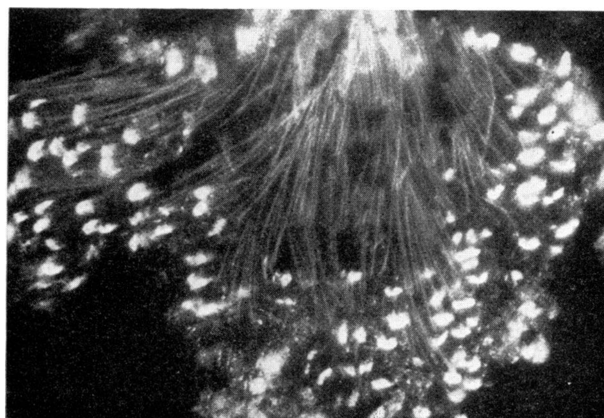

FIG. 4. Rat testis showing fluorescence of spermatozoa. (Magnification ×225.)

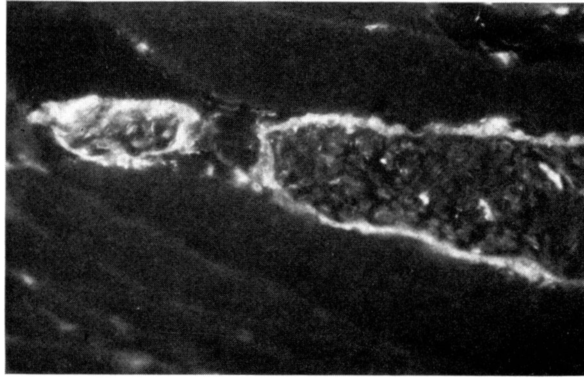

FIG. 5. Rat nerve in skeletal muscle showing peripheral IFL of each nerve fibre and staining of the cell membranes of connective tissue cells and capillary endothelium.

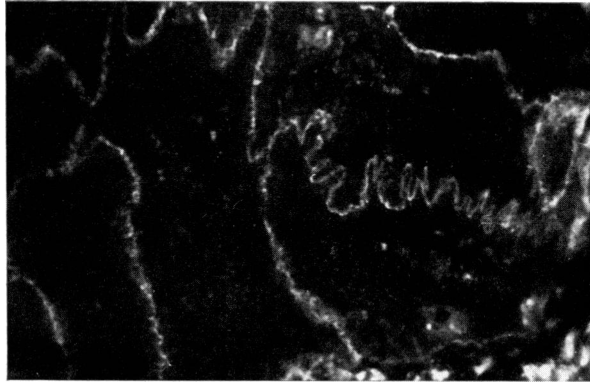

FIG. 6. Ox Purkinje fibres showing staining of the cell membrane and illustrating the complex interdigitations of two adjacent fibres. The beaded appearance reflects increased staining at the tight junctions.

On pancreas all the cells, especially exocrine, were positive as were the cells of the adrenal cortex. The Leydig and sperm cells of rat testis gave striking reactions (Fig. 4). In myelinated nerve fibres the sheaths were strongly positive (Fig. 5). The close relationship of the antigen to the cell membrane was well seen in ox heart Purkinje fibres, where the IFL followed the complex interdigitations of adjacent cells (Fig. 6). Viable lymphocyte suspensions showed no peripheral staining, though these cells became positive when fixed. It was concluded that the antigen was widely distributed in all tissues and situated just below the cell membrane.

The antigen was not denatured by fixation for up to 30 min in acetone, ether, or 1% formaldehyde but was inactivated by ethanol, methanol and glutaraldehyde. The reacting antibody was completely absorbed by the thyroid membrane preparation.

A survey of 20,000 autoantibody results including 10–20% of liver cases, produced only one other serum with a similar wide reactivity in a case of postoperative jaundice following Halothane anaesthesia. In this patient the titre was only 20 and the antibody was no longer present 2 years after recovery.

*Other antibodies present in A.M. serum*

Striational I band IFL of IgG class was seen in cardiac muscle to a titre of 640 later decreasing to 80. Unlike the usual striational antibodies in myasthenia, A.M. serum stained skeletal muscle more weakly than heart (cf Fig. 5 with Fig. 2).

The serum also contained a mixture of diffuse and nucleolar ANA, both entirely of IgM class, present

to a titre of 20 throughout the period of follow-up. These fluorescent patterns were not absorbed out by the cell-membrane preparation and the ANA resisted fixation by ethanol and methanol.

Using the bungarotoxin inhibition assay for antibodies to the acetylcholine receptor site in the neuromuscular junction (Bender *et al.*, 1975), A.M. serum gave a weakly positive reaction (kindly performed by Dr J. Lindström).

## DISCUSSION

The rare antibody found in this case was characterized by its reaction with a plasma membrane-associated component inaccessible to the surface, having a widespread distribution in animal cells, and by its negative reaction with smooth muscle. The antigen was therefore unrelated to contractile proteins although in commonly used substrates such as thyrotoxic thyroid and liver, the pattern was indistinguishable from that obtained with some smooth muscle antibodies especially anti-actin (Gabbiani *et al.*, 1973; Lidman *et al.*, 1976; Bottazzo *et al.*, 1976). Anti-myosin antibody also gives a peripheral IFL (Fairfax, unpublished results). Actin and myosin are known to constitute the network of short contractile fibrils situated just under the plasma membrane of tissue cells (Pollard & Weihing, 1974).

Owing to its rarity, the relationship of the antibody to the clinical condition cannot be established but the two positive sera identified so far came from patients with evidence of liver involvement. Chronic liver disease is only occasionally seen with myasthenia gravis. Simpson (1966) described only two cases in 501 myasthenics in Britain. Other large series reviewed by him contained four further instances amongst 584 MG cases. Alarcón-Segovia *et al.* (1963) described an illness resembling lupoid hepatitis following thymectomy in two cases of MG. Evidence that hepatitis and myasthenia may both result from an inherited dysimmune state was given in a family study by Whittingham, Mackay & Kiss (1970), who described two cases of lupoid type chronic active hepatitis in a family with a case of MG. The association of myasthenia and chronic active hepatitis might be regarded as fortuitous but for the evidence of widespread immunological disorders in myasthenia (Engel *et al.*, 1974). These patients have an unexpectedly high incidence of thyroid diseases, diabetes, rheumatoid arthritis, pemphigus, systemic lupus erythematosus and various malignancies (Simpson, 1960; Osserman & Genkins, 1971). There is an increased prevalence of autoantibodies including striational antibodies to muscle (Strauss *et al.*, 1960), ANA (Feltkamp, 1966) and thyroid antibodies (Simpson, 1966). The description of a serum factor in myasthenia which blocks the acetylcholine receptor sites on human neuromuscular junctions suggests that circulating immunoglobulins may participate in the pathogenesis of the disease (Bender *et al.*, 1975). Occasionally, unusual antibodies have been described in myasthenia for instance anti-U to red cell membranes (Beck *et al.*, 1972) and the present membrane associated antibody is further evidence of a general immunopathological disturbance.

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## REFERENCES

Alarcón-Segovia, D., Galbraith, R.F., Maldonado, J.E. & Howard, F. M. (1963) Systemic lupus erythematosus following thymectomy for myasthenia gravis: report of two cases. *Lancet*, ii, 662.

Beck, M.L., Butch, S.H., Armstrong, W.D. & Oberman, H.A. (1972) An autoantibody with U-specificity in a patient with myasthenia gravis. *Transfusion*, 12, 280.

Bender, A.N., Ringel, S.P., Engel, W.K., Daniels, M.P. & Vogel, Z. (1975) Myasthenia gravis: a serum factor blocking acetylcholine receptors of the human neuromuscular junction. *Lancet*, i, 607.

Biberfeld, G., Fagraeus, A. & Lenkei, R. (1974) Reaction of human smooth muscle antibody with thyroid cells. *Clin. exp. Immunol.* **18**, 371.

Bottazzo, G.F., Christensen, A.F., Fairfax, A.J., Swana, G., Doniach, D. & Groeschel-Stewart, U. (1976) Classification of smooth muscle autoantibodies detected by immunofluorescence. *J. clin. Path.* **29**, 403.

Engel, W.K., Festoff, B.W., Patten, B.M., Swerdlow, M.L., Newball, H.H. & Thompson, M.D. (1974) Myasthenia gravis. *Ann. intern. Med.* **81**, 225.

Feltkamp, T.E.W. (1966) *Idiopathic Autoimmune Diseases.* Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam.

GABBIANI, G., RYAN, G.B., LAMELIN, J-P., VASSALLI, P., MAJNO, G., BOUVIER, C.A., CRUCHAUD, A. & LÜSCHER, E.F. (1973) Human smooth muscle autoantibody. *Amer. J. Path.* 72, 473.

HORNE, C.H.W., BRIGGS, R.D., NICOL, A.G. & HOPKINS, L.M. (1975) Thyroid-cell-membrane antibodies. *Lancet*, ii, 411.

LIDMAN, K., ERIKSSON, U., FAGRAEUS, A. & NORBERG, R. (1974) Antibodies against thyroid cells in *Yersinia enterocolitica* infection. *Lancet*, ii, 1449.

LIDMAN, K., BIBERFELD, G., FAGRAEUS, A., NORBERG, R., TORSTENSSON, R. & UTTER, G. (1976) Anti-actin specificity of human smooth muscle antibodies in chronic active hepatitis. *Clin. exp. Immunol.* 24, 266.

LINTHICUM, D.S. & SELL, S. (1974) Surface immunoglobulin on rabbit lymphoid cells. *Cell. Immunol.* 12, 443.

OSSERMAN, K.E. & GENKINS, G. (1971) Studies in myasthenia gravis: Review of a 20 year experience in over 1200 patients. *Mt Sinai J. Med.* 38, 497.

POLLARD, T.D. & WEIHING, R.R. (1974) Actin and myosin and cell movement. *Crit. Rev. Biochem.* 2, 1.

SIMPSON, J.A. (1960) Myasthenia gravis: A new hypothesis. *Scot. med. J.* 5, 419.

SIMPSON, J.A. (1966) Myasthemia gravis as an autoimmune disease: clinical aspects. *Ann. N.Y. Acad. Sci.* 135, 506.

SMITH, B.R. & HALL, R. (1974) Thyroid stimulating immunoglobulins in Graves' disease. *Lancet*, ii, 427.

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

SUTTON, R.N.P., EMOND, R.T.D., THOMAS, D.B. & DONIACH, D. (1974) The occurrence of autoantibodies in infectious mononucleosis. *Clin. exp. Immunol.* 17, 427.

WHITTINGHAM, S., MACKAY, I.R. & KISS, Z.S. (1970) An interplay of genetic and environmental factors in familial hepatitis and myasthenia gravis. *Gut*, 11, 811.