Clin. exp. Immunol. (1967) **2**, 103–107.

THYMIC GERMINAL CENTRES IN MYASTHENIA GRAVIS: A CORRELATIVE STUDY

G. GOLDSTEIN

Clinical Research Unit, Royal Melbourne Hospital, and the Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia

(*Received* 20 *June* 1966; *accepted* 15 *July* 1966)

SUMMARY

The density of germinal centres in the thymic medulla in twenty-three cases of myasthenia gravis correlated inversely with age but did not correlate with sex, the duration of symptoms of myasthenia gravis, the presence of thymoma, or the result of tests for antibody to striated muscle and thymic epithelial cells.

The number of germinal centres formed in lymphoid tissues during an immune response in man is known to decrease with increasing age and this probably applies to autoimmune reactions. The inverse correlation of thymic germinal centres and age in myasthenia gravis is thus consistent with an autoimmune reaction occurring in the thymus in all cases of myasthenia gravis.

INTRODUCTION

Lymphoid follicles with germinal centres were first recognized in the thymus in myasthenia gravis by Sloan (1943), and Castleman & Norris (1949) showed that this change was present in some 80% of cases. In other cases of myasthenia gravis there is a thymoma, but the thymus adjacent to the thymoma may contain germinal centres (Castleman & Norris, 1949).

Castleman & Norris (1949) found no relationship between the presence of germinal centres in the thymus and the severity of the disease, or the outcome of the disease after thymectomy in their thirty-five cases. This paper examines the relationship between the density of germinal centres in the thymic medulla in myasthenia gravis and other features of the disease.

MATERIALS AND METHODS

Source of thymus

Specimens of thymus were obtained from twenty-three cases of myasthenia gravis, twenty from thymectomy and three from necropsy. The twenty-three patients, twenty females and three males, were aged 14–65 years at the time the thymus was obtained.

Correspondence: Dr Gideon Goldstein, The Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Post Office, Victoria, Australia.

Quantitative study of germinal centres in thymus

The method used to assess the density of germinal centres in the thymic medulla was as described by Goldstein & Mackay (1965). In brief, a slide with a section of thymus was placed on a white background and a 9 mm circle (area 64 mm^2) was so drawn on the cover-slip that as much thymic tissue as possible was included within the circle. The encircled area was projected onto paper at fifteen-fold magnification and the outline of the medulla was drawn on the paper. The outline of the medulla was cut out and weighed so that the area of medulla (in mm^2) within the circle, could be calculated by comparison with the weight of the outline of the 64 mm^2 area similarly projected. The germinal centres in the encircled area were counted, and the density of germinal centres was expressed as the number per mm^2 of thymic medulla. Since in many cases only one section of thymus was available, only one area was scanned for each case.

Antibody to striated muscle and thymus

Serum from ten cases was tested for antibody to skeletal and cardiac muscle and to the large cells in the calf thymic medulla known as 'epithelial cells' using the immunofluorescent indirect sandwich technique (Weller & Coons, 1954; Van der Geld, Feltkamp & Oosterhuis, 1964). Fluorescein labelled goat anti-human γ-globulin (Microbiological Associates, Bethesda, Md., U.S.A.) was used. This had a fluorescein–protein ratio of 3 : 1, a protein content of 10 mg/ml and an antiglobulin–antibody titre of 1 : 16 by agar gel double immuno-diffusion.* Sera were diluted 1 : 10 before testing and no control sera from 100 healthy blood donors showed reactivity to muscle striations and thymic epithelian cells at this dilution.

Statistical methods

A correlation coefficient was calculated between density of germinal centres in the thymic medulla and the age and sex of the patients, the incidence of antibody to striated muscle and thymic 'epithelial cells', the presence of thymoma, and the duration of symptoms of myasthenia gravis. The P value for each of these correlation coefficients was read from a table of correlation coefficients.

RESULTS

The clinical data including sex and age of the patients and duration of symptoms of myasthenia gravis, serological findings, and density of medullary germinal centres are presented in Table 1. The serum of four of ten cases tested contained antibody to striations of skeletal muscle and thymic 'epithelial cells'; the other six cases had neither antibody.

The density of medullary germinal centres which ranged from 0 to 1·73 per mm^2 of medulla correlated inversely with the age of the patient, the correlation coefficient (CC) being $-0{\cdot}548$ (Fig. 1). For 21 degrees of freedom (N) this inverse correlation is highly significant ($P<0{\cdot}01$). Thus for the series as a whole the thymus of older patients contained few or no germinal centres whereas the thymus of younger patients contained many. The finding that the thymus of two younger patients (Cases 9 and 10) contained no germinal centres may be explained by sampling, since only one section of thymus was examined

* Information from suppliers.

from each case. The density of germinal centres did *not* correlate ($P > 0{\cdot}10$) with: (i) the sex of the patient (CC $-0{\cdot}201$, N 21), (ii) the duration of symptoms of myasthenia gravis (CC $-0{\cdot}020$, N 21), (iii) the presence of a thymoma (CC $-0{\cdot}015$, N 21), and (iv) the result of the test for antibody to striated muscle and thymic 'epithelial cells' (CC $-0{\cdot}490$, N 8).

TABLE 1. Clinical, histopathological and serological data on twenty-three patients with myasthenia gravis

Case number*	Age (years) at time of obtaining thymus	Density of germinal centres (No. per mm² medulla)	Antibody to striated muscle and thymic 'epithelial cells' †	Duration of symptoms of myasthenia gravis (months)
1	14	1·11	n.t.	2
2	17	1·73	neg	18
3	18	1.01	neg	6
4	20	0·58	neg	30
5	21	0·97	neg	12
6	23	0·67 T	pos	31
7	26	0·32	n.t.	12
8	26	0·26	n.t.	9
9	27	0	n.t.	10
10	27	0	n.t.	12
11	28	0·41	n.t.	4
12	41	0	n.t.	8
13	42	0	n.t.	15
14	44	0·75 T	n.t.	10
15	45	0·50	neg	12
16	52	0·63	n.t.	14
17	52	0·68	n.t.	84
18	52	0·36	n.t.	312
19	56	0	neg	12
20	56	0	n.t.	8
21	58	0·44 C	pos	22
22	65	0 T	pos	6
23	65	0	pos	41

T = thymoma; C = cyst. n.t. = not tested.

* Cases 6, 20 and 22 were male. Cases 12, 18 and 19 were necropsy specimens, remainder obtained at thymectomy.

† Positive cases showed concurrent reactivity with both striated muscle and thymic 'epithelial cells'

DISCUSSION

Germinal centres appear in lymphoid tissue during immunological responses and also in lymphoid infiltrates in various tissues in states associated with autoimmune reactions (Burnet & Mackay, 1962). Germinal centres are seldom present in the normal thymus and their presence in the thymus in myasthenia gravis is interpreted as evidence for an immunological reaction in the thymus (White & Marshall, 1962). Moreover the finding of an antibody

to striated muscle and thymic 'epithelial cells' in the serum of patients with myasthenia gravis (Strauss *et al.*, 1966) suggests that the germinal centre reaction in the thymus is an autoimmune response to thymic antigen.

Germinal centres are reactive structures normally produced in lymphoid tissues in response to immunological stimuli by extrinsic antigen. The degree of germinal centre formation in lymphoid tissues in man is maximal at puberty and decreases with increasing age (Denz, 1947): presumably the formation of germinal centres in response to self antigens would also decrease with age. This in fact was the finding in the present study. Thus the density of germinal centres in the thymus in these twenty-three cases did not correlate with either sex,

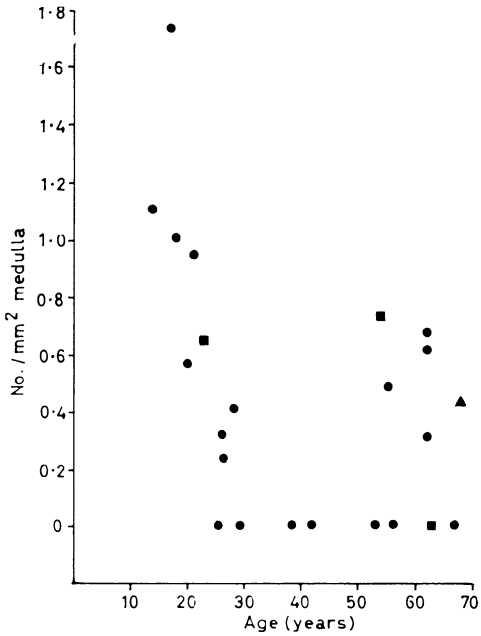

FIG. 1. Scattergram to show the corresponding values of density of germinal centres in the medulla and the age of the patient in twenty-three cases of myasthenia gravis. Associated thymic pathology: ●, none; ■, thymoma; ▲, cyst. The correlation coefficient was −0·548 ($P<0·01$). The highly significant inverse correlation between these indices shows that the density of germinal centres in the thymic medulla in cases of myasthenia gravis decreases with increasing age.

duration of symptoms, thymoma, or the presence of the anti-striated muscle antibody, but did correlate with the age of the patient—the older the patient, the fewer the germinal centres. Thus scarcity or apparent absence of germinal centres in the thymus, especially in older patients, is not inconsistent with the concept that an autoimmune reaction occurs in the thymus in all cases of myasthenia gravis.

ACKNOWLEDGMENTS

I am grateful to Dr Ian R. Mackay for his stimulating interest in this work and to Dr Senga Whittingham for the serological studies. Specimens were received through the kind courtesy

of Dr J. Funder, Dr R. Greene, Mr J. Hayward, Dr J. D. Hicks, Mr I. McConchie, Dr A. Schwieger, Mr G. Stirling and Dr A. Tait Smith. Mr A. Bargerbos provided excellent technical assistance. The author is in receipt of a grant from the National Health and Medical Research Council of Australia.

REFERENCES

BURNET, F.M. & MACKAY, I.R. (1962) Lymphoepithelial structures and autoimmune disease. *Lancet*, **ii,** 1030.

CASTLEMAN, B. & NORRIS, E.H. (1949) The pathology of the thymus in myasthenia gravis: A study of 35 cases. *Medicine (Baltimore)*, **28,** 27.

DENZ, F.A. (1947) Age changes in lymph nodes. *J. Path. Bact.* **59,** 575.

GOLDSTEIN, G. & MACKAY, I.R. (1965) Contrasting abnormalities in the thymus in systemic lupus erythematosus and myasthenia gravis: A quantitative histological study. *Aust. J. exp. Biol. med. Sci.* **43,** 381.

SLOAN, H.E., JR. (1943) The thymus in myasthenia gravis, with observations on normal anatomy and histology of the thymus. *Surgery*, **13,** 154.

STRAUSS, A.J.L., SMITH, C.W., CAGE, G.W., VAN DER GELD, H.W.R., MCFARLIN, D.E. & BARLOW, M. (1966) Further studies on the specificity of presumed immune associations of myasthenia gravis and consideration of possible pathogenic implications. *Ann. N. Y. Acad. Sci.* **135,** 557.

VAN DER GELD, H.W.R., 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.

WELLER, T.H. & COONS, A.H. (1954) Fluorescent antibody studies with agents of varicella and herpes zoster propagated *in vitro*. *Proc. Soc. exp. Biol. (N. Y.)*, **86,** 789.

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