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Immunohistological patterns of non-neoplastic changes in the thymus in Myasthenia gravis*

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Summary. Non-neoplastic thymuses from 20 patients with myasthenia gravis (MG) have been studied by routine stains on paraffin sections and by immunohistological methods on frozen sections using a panel of monoclonal antibodies against thymic epithelial cells, macrophages/reticulum cells, lymphoid cells and myoid cells. Three types of thymic histology in MG were distinguished: (1) thymitis with lymphoid follicular hyperplasia (11 cases), (2) thymitis with diffuse B-cell infiltration (5 cases) and (3) thymic atrophy (4 cases). Thymitis was more common in younger females and thymic atrophy in older patients.

Both types of thymitis were associated with conspicious structural disturbance of the thymic perivascular space (PVS) and medulla, characterized by a distinct enlargement of the PVS and disruption of the epithelium and reticulin fibre network at the medullary boundary, leading to fusion of the two compartments. The PVS and medulla contained a striking B-cell infiltration. Large well-developed germinal centers (GCs), showing the same cellular organization as in the peripheral lymphatic system, occurred in thymitis with lymphoid follicular hyperplasia, whereas thymitis with diffuse B-cell infiltration merely exhibited a few tiny lymphoid follicles, which could be demonstrated only by immunostaining of dendritic reticulum cells. In thymic atrophy a diffuse B-cell infiltration of the PVS and the medulla was also observed, but only minor alterations of the epithelial framework were seen.

There was an increased number of interdigitating reticulum cells with variable expression of the T-6 antigen in all the thymuses examined, indicating an immune stimulation of the intrathymic T-cells. Myoid cells, the supposed target of the intrathymic immune reaction in MG, were

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found to be less frequent in thymic atrophy than in thymitis. This variable number of myoid cells may explain the different grades of immune stimulation and different types of histology seen in the thymus in MG.

Key words: Thymus – Myasthenia gravis – Immunohistology

Introduction

Myasthenia gravis (MG) is an autoimmune disease mediated by autoantibodies to the nicotinic acetylcholinereceptor (AchR) of striated muscle. Abnormalities of the thymus are associated with muscle weakness in the majority of patients. About 10% of patients with MG have a thymic epithelial tumor; of the remainder 65 to 80% exhibit germinal centres (GCs) in the thymus (Castleman and Norris 1949; Levine and Rosai 1978; Otto 1984).

New immunological findings in the last few years have underlined the connection between thymic alterations and the pathogenesis of MG. It has been shown that intrathymic myoid cells, which possess ultrastructural features of striated muscle (Drenckhahn et al. 1979; Palestro et al. 1983), express surface AchRs in tissue culture (Kao and Drachman 1977; Wekerle et al. 1978). In addition, thymic cell suspensions from patients with MG, which appear to be derived mainly from GCs, spontaneously synthesize anti-AchR-antibodies (Scadding et al. 1981; Willcox et al. 1983). Recently T-cell lines with anti-AchR reactivity have been produced from the thymuses of patients with MG (Melms et al. 1986). Further evidence in support of the possible mechanism of autoimmunization in MG is offered by studies of a structural disorganization of thymic microenvironments using monoclonal antibodies (Janossy et al. 1986; von Gaudecker 1986; Wekerle and Müller-Hermelink 1986).

Previous immunohistological investigations of non-neoplastic thymic changes in MG have focussed mainly on cases with well-developed intrathymic GCs, and thymuses without GC formation or with involution have not usually been considered (Scadding et al. 1981; Thomas et al. 1982; Pizzighella et al. 1983; Palestro et al. 1983; Kornstein et al. 1984; Bofill et al. 1985). However, analysis of the different morphological findings in the thymus may be essential in understanding the clinical and immunological heterogeneity of MG.

Compston et al. (1980) have pointed out that MG patients without thymoma can be divided into two groups. The majority of cases presenting before the age of 40 years have thymic lymphoid follicular hyperplasia, characterized by pronounced intrathymic GC formation. In this first group females predominate and the frequencies of HLA-A1, B8 and DRw3 are significantly increased. In the second group the thymus is usually involuted; these patients are predominantly male, present after the age of 40 years and show increased frequencies of HLA-A3, B7 and DRw2.

We studied non-neoplastic thymuses from 20 patients with MG, aged between 2 and 72 years, including cases without GCs and with thymic atrophy, and we have distinguished three different immunohistological patterns.

Material and methods

Material. Fresh thymuses were obtained from 20 patients with MG undergoing thymectomy. Only cases without thymomas were included in this study. The diagnosis of MG was based on the clinical findings and included electromyographic criteria and a determination of anti-AchR-antibodies and anti-striated-muscle-antibodies (Table 2).

Controls. The study was based on previous investigations of normal thymic histology and immunohistology in patients without systemic disease. This material was obtained at autopsy in 136 patients dying suddenly and from fresh thymic tissue from 85 patients undergoing cardiac surgery; the patients were aged between 0 and 107 years (Müller-Hermelink et al. 1982; Müller-Hermelink and Steinmann 1984; Steinmann and Müller-Hermelink 1984). The reactivity patterns of the antibodies used in this study had been examined previously in the normal control material.

Routine histology. Paraffin sections (5 μm thick) from formalin-fixed specimens were stained with H & E, Giemsa, PAS and silver stains.

Immunohistology on frozen sections. Fragments of fresh thymus were snap-frozen in liquid nitrogen. Cryostat sections (5 μm thick) were fixed in acetone for 10 min at room temperature and air-dried.

Except for the monoclonal anti-desmin antibody, all immunohistochemical reactions were performed by the indirect immunoperoxidase technique using a three-stage procedure (Stein et al. 1982b). The incubation steps were the following: I) purified monoclonal antibody (Table 1) for 30 min, II) peroxidase-conjugated rabbit anti-mouse immunoglobulins (DAKO, Copenhagen, Denmark) for 30 min, III) peroxidase-conjugated goat anti-rabbit IgG antibody (Medac, Hamburg, FRG) for 30 min. Sections were then incubated for 10 min with diaminobenzidine (DAB) (Sigma, München, FRG) and the peroxidase activity was demonstrated according to Graham and Karnovski (1966). After every incubation step with antibody and with DAB, sections were washed with TRIS (Tris-hydroxy-methyl-aminomethane) buffer (pH 7.4). Slides were counterstained with haemalum.

The immunohistochemical reaction for the monoclonal anti-desmin antibody was performed by the alkaline phosphatase method (Feller et al. 1983). The incubation steps were the following: I) purified monoclonal anti-desmin antibody (Laboserv GmbH, Giessen, FRG), diluted 1:5, for 30 min, II) rabbit anti-mouse immunoglobulins (DAKO, Copenhagen, Denmark), III) alkaline phosphatase-conjugated goat anti-rabbit IgG antibody (Sigma, München, FRG), IV) alkaline phosphatase reaction according to a modified version of the method described by Kaplow (1968) and Lojda et al. (1976). Enzyme histochemical reaction was achieved by a combination of Naphthol-AS-BI-phosphate (Sigma, München, FRG) as substrate and Fast Red TR (Sigma, München, FRG) as coupler for the staining of exogenous alkaline phosphatase. The substrate and the coupler were dissolved in N,N-dimethylformanide (DMF; Merck, Darmstadt, FRG) (50 mg/ml). The coupler was diluted with 50 ml 0.05 M propandiol buffer pH 9.75 and the final pH was adjusted to 9.4. Incubation media were supplemented with 1 mM levamisole (Sigma, München, FRG) for the inhibition of endogenous tissue enzyme activity (Ponder and Wilkinson 1981). The slides were incubated for 10 min at room temperature. After every incubation step with antibody and after the alkaline phosphatase reaction, the slides were rinsed with TRIS buffer (pH 7.4). Slides were counterstained with haemalum. For control of non-specific binding, the primary antibody was replaced by phosphate-buffered saline (PBS).

The immunohistological patterns of the thymuses from MG patients were evaluated in parallel sections so that the reactivity of the antibodies used could be compared in each case. Sections of normal thymus were stained in parallel with the thymuses from MG patients to control the localization and the amount of the immunohistochemical reaction product.

Immunohistology on paraffin sections. Demonstration of desmin was performed on 5 μm thick sections of formalin-fixed, paraffin-embedded thymic fragments by an immunoperoxidase technique using a polyclonal antidesmin antibody and the avidin-biotin-peroxidase complex method

Table 1. List of antibodies used

Antibody	Clones or producer animal	mc[a] pc	Antigen or cells identified	Source
Anti-HLA-A, B, C	61D2	mc	Major serogically defined antigens HLA-A, B and C	Bethesda Research Laboratories, Neu-Isenburg, FRG
Anti-HLA-DR	7.2	mc	HLA-DR antigens (Ia-like; p 28, 33)	New England Nuclear, Dreireich, FRG
35βH11	Balb/c mouse	mc	54kd[b] keratin(s) in most non-squamous epithelia	Gown and Vogel, J Cell Biol 95, 414, 1982
34βE12	Balb/c mouse	mc	57/66kd[b] keratins in squamous epith., ductal epith., parabasal glandular cells	Gown and Vogel, J Cell Biol 95, 414, 1982
IV/82	Balb/c mouse	mc	Squamous epithelium keratin	Pathology Institute, Kiel, FRG
Ki-M3	Balb/c mouse	mc	Monocytes and subpopulations of macrophages; all thymic epithelial cells	Radzun and Parwaresch, Cell Immunol, 82, 174, 1983
Ki-M1	Balb/c mouse	mc	Monocytes and macrophages including interdigitating reticulum cells	Radzun and Parwaresch, Cell Immunol, 82, 174, 1983
Ki-M6	Balb/c mouse	mc	Monocytes and macrophages	Dr. Radzun, Kiel, FRG
Ki-M4	Balb/c mouse	mc	Dendritic reticulum cells	Parwaresch et al., Blood 62, 585, 1983
Anti-Leu-1	L17F12	mc	Human T-cell antigen (Mr 67K daltons)	Becton-Dickinson, Oxnard, Calif., USA
Anti-Leu-2a	SK1	mc	Suppressor/cytotoxic subset of T-cells	Becton-Dickinson, Oxnard, Calif., USA
Anti-Leu-3a	SK3	mc	Helper/inducer subset of T-cells	Becton-Dickinson, Oxnard, Calif., USA
Anti-Leu-4	SK7	mc	Human T-cell antigen (Mr 20K–30K daltons)	Becton-Dickinson, Oxnard, Calif., USA
OKT 3	OKT 3	mc	Human T-cell antigen	Ortho Diagnostic System, Inc, Raritan, New Jersey, USA
OKT 11	OKT 11	mc	Sheep erythrocyte receptor-positive T-cells	Ortho Diagnostic System, Inc, Raritan, New Jersey, USA
Anti-Leu-6	SK9	mc	Human common thymocytes	Becton-Dickinson, Oxnard, Calif., USA
OKT 6	OKT 6	mc	Human common thymocytes	Ortho Diagnostic System, Inc, Raritan, New Jersey, USA
Anti-Leu-7	HNK-1	mc	Human lymphocyte antigen in natural killer/killer cells; thymic surface epithelium	Becton-Dickinson, Oxnard, Calif., USA
To 15	To 15	mc	All B-cells	Dako, Copenhagen, Denmark
Anti-Desmin		mc	Desmin intermediate filaments	Laboserv, Giessen, FRG
Anti-Desmin		pc	Desmin intermediate filaments	Laboserv, Giessen, FRG

[a] mc: monoclonal; pc: polyclonal
[b] molecular weight of keratin polypeptide

(Hsu et al. 1981). After blocking endogenous peroxidase activity with methanol containing 1% H_2O_2 for 25 min, the following incubation steps were performed in an incubation chamber (Ormanns and Pfeifer 1981) at room temperature: I) polyclonal anti-desmin antibody, diluted 1:25, for 16 h, II) biotin-conjugated goat anti-rabbit antibody for 30 min, III) peroxidase conjugated biotin-avidin complex for 30 min. The DAB reaction of Graham and Karnovsky

(1966) was used for visualizing peroxidase activity. After every incubation step and the DAB reaction, the slides were rinsed with PBS (pH 7.4). Slides were counterstained with haemalum. For control of non-specific binding, the primary antibody was replaced by PBS.

Results

Types of histological change in the non-neoplastic thymus in MG

Pronounced GC formation, the most frequent thymic alteration in MG (Levine and Rosai 1978), was seen in 11 cases in this study. The change was originally called 'lymphoid follicular hyperplasia of thymus' by Castleman and Norris (1949). In view of the structural alterations of thymic microenvironment, which are interpreted as sequelae of an intrathymic inflammatory process, we prefer the term 'thymitis with lymphoid follicular hyperplasia'.

In nine of our cases no thymic GCs were found in routine stains (H & E, Giemsa). Of these, in five the immunohistological findings revealed an abnormal intrathymic immune reaction with structural alterations and a diffuse B-cell infiltration, but only very few, tiny lymphoid follicles. Accordingly we called this change 'thymitis with diffuse B-cell infiltration'. In the remaining four cases the thymus showed involution and only minor immunohistological alterations. This change was described as 'thymic atrophy'.

Table 2 lists the age and sex of the patients as well as the clinical findings in the three types of non-neoplastic thymic changes in MG.

Thymitis with lymphoid follicular hyperplasia in MG

Routine histology. The characteristic feature of thymitis with lymphoid follicular hyperplasia is pronounced intrathymic GC formation clearly evident in routine stains (H & E, Giemsa). The number of GCs varies; sometimes only a few GCs are found, but more often GCs are evenly distributed or focally aggregated.

Silver staining allows the distinction of cortex and medulla from the perivascular space (PVS), defined by its dense network of reticulin fibres. In thymitis with lymphoid follicular hyperplasia the PVS becomes distended, starting with a club-shaped enlargement at the corticomedullary junction. The number of reticulin fibres increases in the PVS as well as in the medulla, so that in some thymuses the PVS and medulla are no longer distinguishable by silver staining. Small vessels proliferate and branch into the medulla, and in the neighbourhood of GCs epitheloid venules with frequent lymphocyte diapedesis are found. The cortex shows a normal width and no increase in reticulin fibres.

Immunohistology. The cytological and immunohistological features of intrathymic GCs do not differ from lymphoid follicles in peripheral lymphatic organs, as described by Stein et al. (1982a) and Lennert and Stein (1982). Accordingly the intrathymic GCs are derived from B-cells, marked by the

Table 2. Clinical data and thymic histology in 20 patients with MG

Case	Sex	Age (years) at thymectomy	Duration of symptoms (years)	Drug treatment prior to thymectomy	Clinical grade[a]	Anti-AchR-antibodies	Anti-striated-muscle antibodies	Electrophysiological criterias	Type of thymic histology[b]
1	M	2	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	I
2	M	16	0.5	Pyridostigmine	IIa	+	−	Decr.+[c]	I
3	F	17	1	Pyridostigmine	IIb	+	+	Decr.+	I
4	F	19	2	Pyridostigmine	IIb	+	−	Decr.+	I
5	F	22	1	Pyridostigmine	IIb	N.d.[d]	+	Decr.+	I
6	F	23	0.7	Pyridostigmine	IIb	N.d.	N.d.	Curare-test+	I
7	F	24	6	Pyridostigmine	IIb	+	N.d.	Decr.+	I
8	M	27	0.7	Prednisone	IIb	+	+	Tensilon-test+	I
9	F	29	10	Pyridostigmine	IIa	+	N.d.	Decr.+	I
10	F	36	2.5	Pyridostigmine	IIb	+	N.d.	Decr.+	I
11	F	39	2.2	None	IIa	N.d.	+	Decr.+	I
12	F	12	1	Prednisone Azathioprine	III	+	N.d.	Decr.+	II
13	F	15	0.5	Pyridostigmine	IIa	+	+	Curare-test+ Tensilon-test+	II
14	F	26	6	Azathioprine Pyridostigmine	IIa	−	+	Decr.+	II
15	F	33	9	Azathioprine Prednisone Pyridostigmine	III	−	−	Decr.+	II
16	M	34	0.2	Pyridostigmine	IIa	−	+	Decr.+	II
17	M	30	0.5	None	IIa	−	+	Decr.+	III
18	M	43	0.3	Pyridostigmine	III	+	+	Decr.+	III
19	M	58	2	Pyridostigmine	III	+	(+)	Decr.+	III
20	F	72	0.5	None	IIa	+	+	Decr.+	III

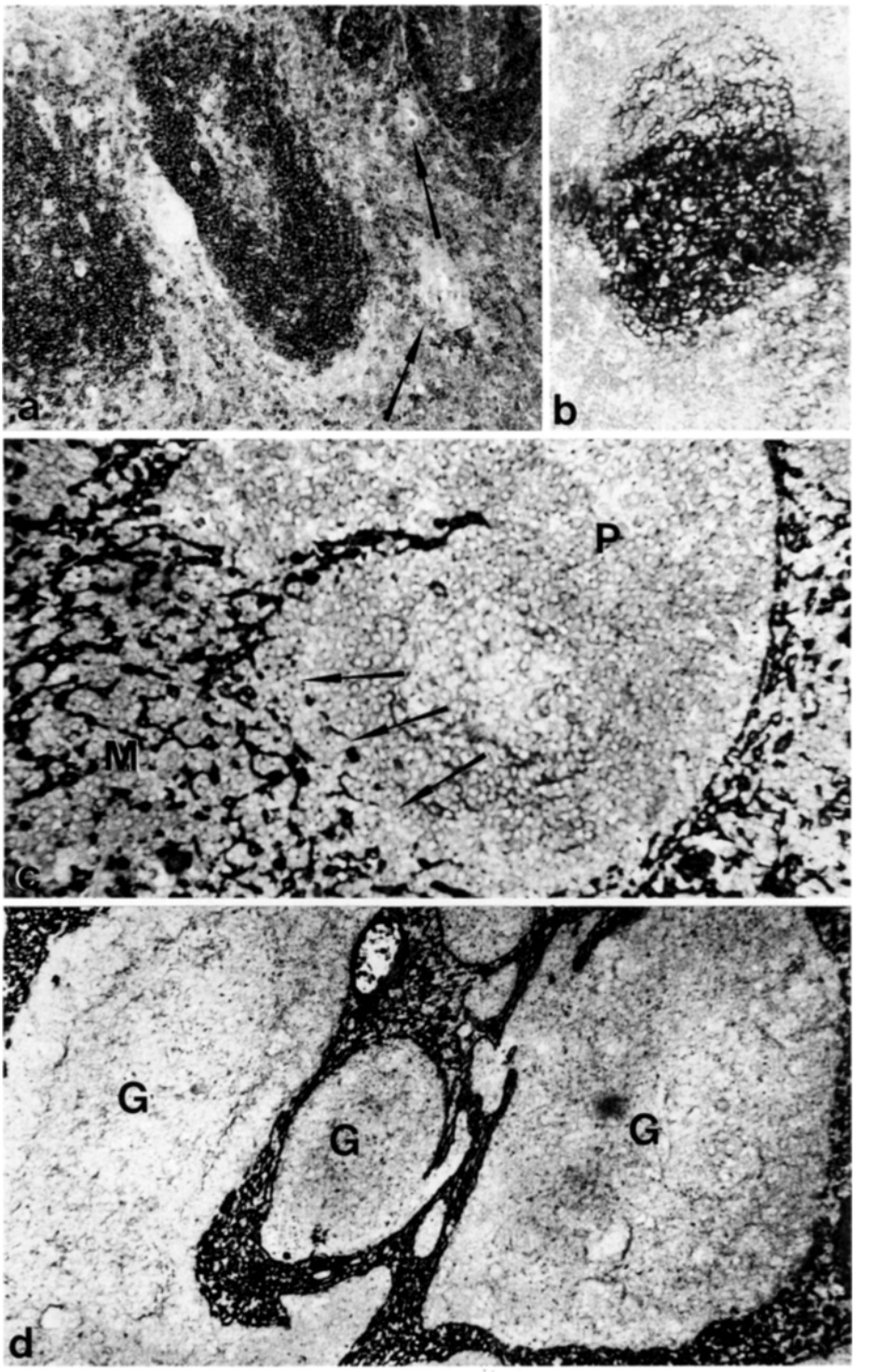
[a] According to Osserman and Genkins (1971). IIa mild; IIb moderate; III severe, generalized symptoms

[b] Type I: Thymitis with lymphoid follicular hyperplasia. Type II: Thymitis with diffuse B-cell infiltration. Type III: Thymic atrophy

[c] Positive decrement (amplitude 1 to 5 larger than 15%) in repetitive nerve stimulation performed in deltoid muscle with stimulation frequency 3/sec

[d] Not determined

Fig. 1a–d. Immunohistological findings in thymitis with lymphoid follicular hyperplasia in MG. **a** Demonstration of lymphoid follicles adjacent to HCs (*arrow*) in the thymic medulla by the antibody To 15. ×95. **b** Positive reactivity of dendritic reticulum cells in a thymic GC with the antibody Ki-M4. ×150. **c** Disruptions of the medullary (M) epithelial cells (*arrows*) at the boundary with the PVS (*P*), as demonstrated by the antibody 35βH11. ×240. **d** The antibody 34βE12 against keratins showing condensed cords of medullary epithelial cells between the extremely extended epithelium-free PVS. One of the GCs (*G*) is completely enclosed by the medullary epithelium. ×95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antibody To 15 (Fig. 1a), and contain dendritic reticulum cells with a selective expression of the Ki-M4 antigen (Fig. 1b).

In addition to GC formation there is a pronounced, diffuse B-cell infiltration of the PVS and medulla, and focal B-cell accumulation around Hassalls corpuscles (HCs) demonstrated by immunostaining with the antibody To 15. These diffuse B-cell infiltrates as well as the GCs are always restricted to the PVS and the medulla and the cortex is not involved.

Considerable structural disturbances of the PVS and medulla are confirmed by immunostaining of thymic epithelial cells with different anti-keratin antibodies (IV/82, 34βE12, 35βH11) and with the antibody Ki-M3. At the border between the PVS and the medulla, disruption of the epithelial barrier occurs (Fig. 1c). Sometimes small medullary epithelial islands are completely surrounded by the extended PVS. Although GC formation predominates in the enlarged PVS, some GCs are confined to the medulla and enclosed by the medullary epithelium on all sides (Fig. 1d). Cords of medullary epithelial cells are condensed between the GCs in a manner reminiscent of the structural relationship between the crypt epithelium and lymphoid follicles in the tonsil (Fig. 1d).

In most cases the medulla shows strong HLA-DR expression by the epithelial cells, macrophages/reticulum cells and lymphocytes, which is comparable to the HLA-DR pattern of normal thymus (Steinmann 1986), but sometimes there are small medullary foci exhibiting a weaker or almost absent HLA-DR expression by epithelial cells and lymphocytes. The staining pattern for HLA-A, B and C antigens is similar to that observed in the normal thymus (Steinmann 1986).

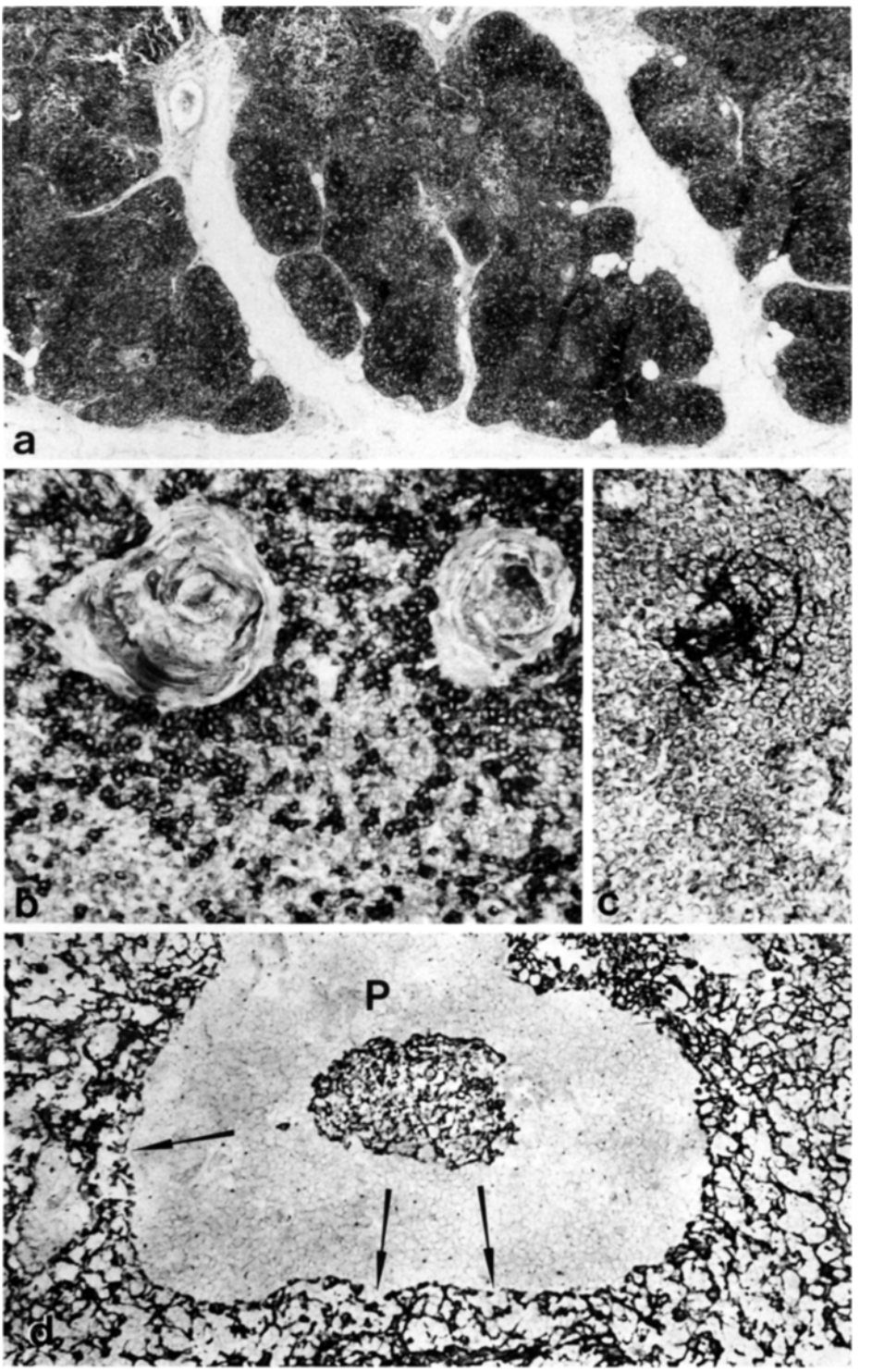
The intrathymic distribution of different T-cell subpopulations in thymitis with lymphoid follicular hyperplasia does not differ significantly from the findings in the normal thymus described by Janossy et al. (1981) and by Steinmann (1986).

Occasional Leu 7-positive natural killer/killer cells may also be found in the medulla, whilst in the cortex, very few, if any, Leu 7-positive lymphoid cells occur. As in the normal thymus (Bofill et al. 1985) Leu 7 is expressed by the thymic surface epithelium.

Thymitis with diffuse B-cell infiltration in MG

Routine histology. GCs are not detected by routine stains (HE, Giemsa) (Fig. 2a), whereas the structural disturbance and fusion of the PVS and

Fig. 2a–d. Histological and immunohistological findings in thymitis with diffuse B-cell infiltration in MG. **a** Thymus of a 12-year-old female patient who was treated prior to thymectomy with azathioprine. No GCs are visible by routine staining. HE, ×30. **b** Immunostaining of diffuse B-cell infiltrates around HCs in the thymic medulla (antibody To 15). ×190. **c** Dendritic reticulum cells of a small lymphoid follicle marked with the antibody Ki-M4. ×240. **d** The antibody 35βH11 against keratins exhibiting an isolated group of medullary epithelial cells between the enlarged epithelium-free PVS (*P*). Small epithelial gaps occur at the boundary with the medulla (*arrows*). ×190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medulla resembles the patterns found in thymitis with lymphoid follicular hyperplasia. A conspicious enlargement and club-shaped widening of the PVS is seen in silver stains. The number of reticulin fibres and small vessels is increased in the PVS and in the medullary areas. As in the thymuses with pronounced GC formation, the age-appropriate width and integrity of the thymic cortex appears to be unchanged.

Immunohistology. A constant immunohistological finding in this type of thymitis is the distinct, diffuse B-cell infiltration of the PVS and the medulla shown by the antibody To 15 (Fig. 2b). Ring-like accumulations of B-cells occur around HCs. Very few, tiny lymphoid follicles are found only by immunohistological methods and characterized by a sparce network of Ki-M4-positive dendritic reticulum cells (Fig. 2c). The cortex contains extremely few, if any, B-cells.

Immunostaining of epithelial cells by the antibodies IV/82, 34βE12, 35βH11 and Ki-M3 demonstrates a structural disturbance of medulla and PVS similar to that in thymitis with lymphoid follicular hyperplasia. Although GCs are inconspicious, areas with distinct enlargement of the epithelium-free PVS are found (Fig. 2d). There are gaps in the epithelium at the border between the PVS and medulla and zones with large-meshed splitting of the medullary epithelium also occur. Condensed cords of the medullary epithelial cells are not as pronounced as in thymuses with well-developed GCs.

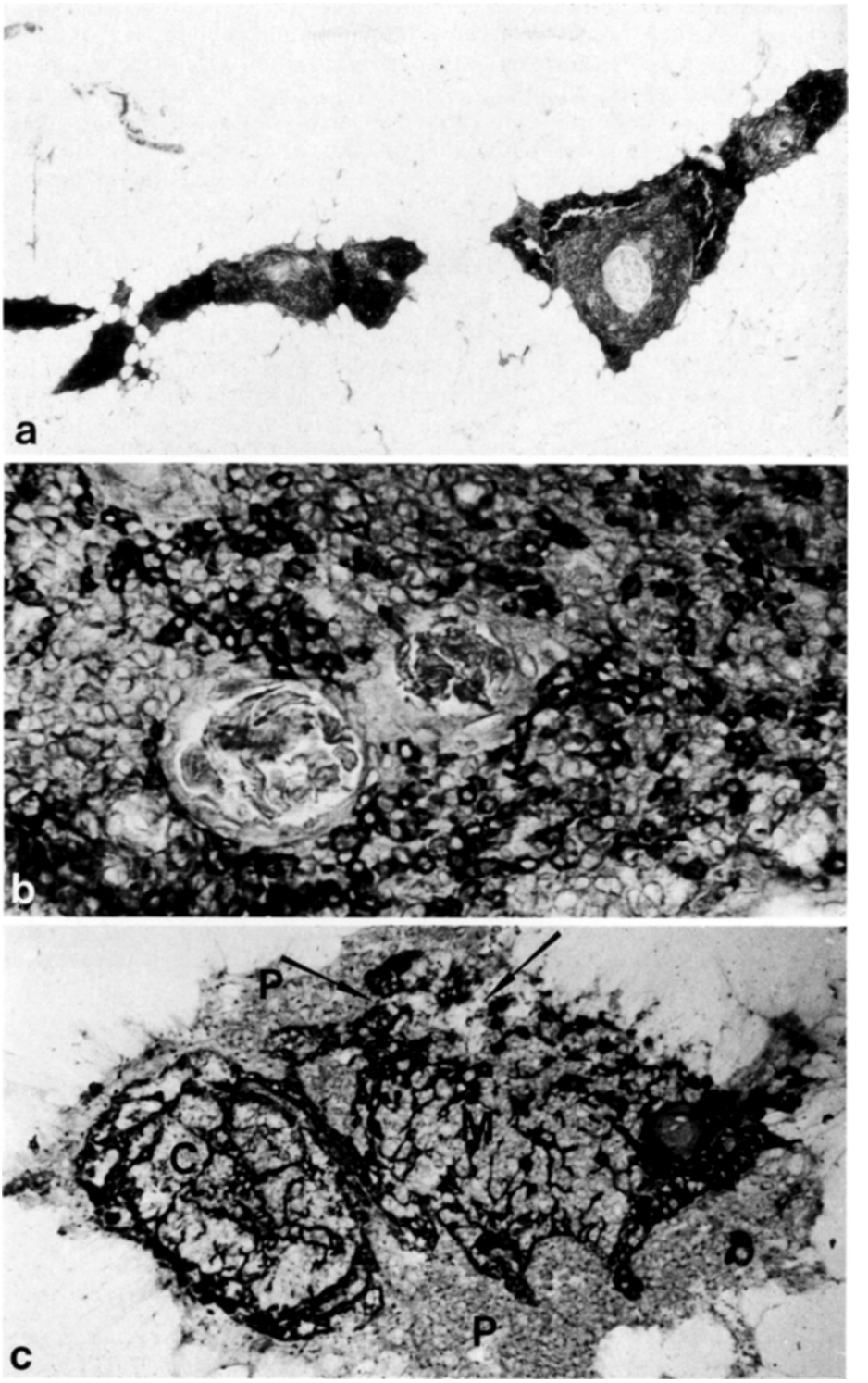
The HLA-DR and HLA-A, B and C expression of epithelial cells and lymphocytes resembles that in thymitis with lymphoid follicular hyperplasia. The intrathymic distribution of T-cell subpopulations is the same as in the normal thymus (Steinmann 1986).

Thymic atrophy in MG

Routine histology. This change is characterized by thymic involution (Fig. 3a). Between the prevailing adipose tissue only small cords and islands of thymic parenchyma are found. These still contain functioning cortical and medullary compartments with a typical lymphocyte distribution and GCs are not demonstrated. The reticulin fibres defining the PVS show only minor extensions, and there is focally discrete splitting of the reticulin fibre network at the medullary boundary. The cortical zones are unchanged.

Immunohistology. There is a pronounced diffuse B-cell infiltration of the PVS and the medulla, verified by immunostaining with the antibody To 15 (Fig. 3b). Only one thymus contained a tiny lymphoid follicle with a few

Fig. 3a–c. Histological and immunohistological findings in thymic atrophy in MG. **a** Thymus of a 30-year-old male patient showing involution and no GCs. HE, ×30. **b** Immunostaining of intramedullary B-cell infiltrates around HCs by the antibody To 15. ×375. **c** Demonstration of the cortical (*C*) and medullary (*M*) epithelium by the antibody 35βH11 against keratins. There are small epithelial disruptions (*arrows*) at the boundary with the epithelium-free PVS (*P*) and medullary epithelium is loosened. ×190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Ki-M4-positive dendritic reticulum cells. Very occasional B-cells are seen in the cortex. In areas of B-cell infiltration the medullary epithelium, marked by the antibodies IV/82, 34βE12 and 35βH11, is moderately dispersed and has some gaps at the boundary of the PVS (Fig. 3c). At the same time a moderate widening of the epithelium-free PVS is observed.

The HLA-DR- and HLA-A, B and C-staining patterns and the distribution of T-cell differentiation antigens resemble the findings in the normal thymus (Steinmann 1986).

Appearance and distribution of thymic interdigitating reticulum cells (IDCs) in MG

The IDCs of human thymus can be characterized by their positive immunostaining with the antibody Ki-M1 and their negative reactivity with the antibody Ki-M6. Since the antibodies Ki-M1 and Ki-M6 are also marking different types of macrophages, parallel sections have to be studied for the identification of IDCs.

In thymitis with lymphoid follicular hyperplasia or with diffuse B-cell infiltration there are increased numbers of IDCs (Fig. 4a) localized in the medulla and particularly around HCs and at the cortico-medullary junction. A variable number of IDCs express the T-6-antigen (Leu 6- and OKT 6-positive) in contrast to the findings in the normal thymus. These T-6-positive IDCs are frequently seen in the immediate neighbourhood of HCs (Fig. 4b). Even in thymic atrophy an increase in IDCs, sometimes expressing the T-6-antigen, is observed.

Multinucleated cells in MG

In routine stains single large round cells with a rim of nuclei near the cell surface are identified at the corticomedullary junction (Fig. 5a). Immunohistochemically, these cells are Ki-M1- and Ki-M6-positive (Fig. 5b and 5c), whereas the reactions for epithelial antigens (IV/82, 34βE12 and 35βH11) are negative. We found multinucleated cells in six of the 11 cases of thymitis with lymphoid follicular hyperplasia and in three of the five cases of thymitis with diffuse B-cell infiltration. Such large cells were absent in thymic atrophy.

Epithelial structure of the thymic cortex in MG

In the normal thymus and in the thymus in MG, cortical epithelial cells give positive immunohistochemical reactions, with the antibodies 35βH11,

Fig. 4a–b. IDCs in the thymus in MG. **a** Demonstration of a large number of macrophages and IDCs (*arrows*) with processes encircling lymphocytes in the medulla by the antibody Ki-M1. × 240. **b** Positive reactivity of some IDCs adjacent to a HC with the antibody OKT 6. × 308

Fig. 5a–c. Multinucleated cells in the thymus in MG. **a** Large cell with a rim of nuclei near the cell surface. HE, × 600. **b** Immunostaining by the antibody Ki-M6. × 600. **c** Immunostaining by the antibody Ki-M1. × 600

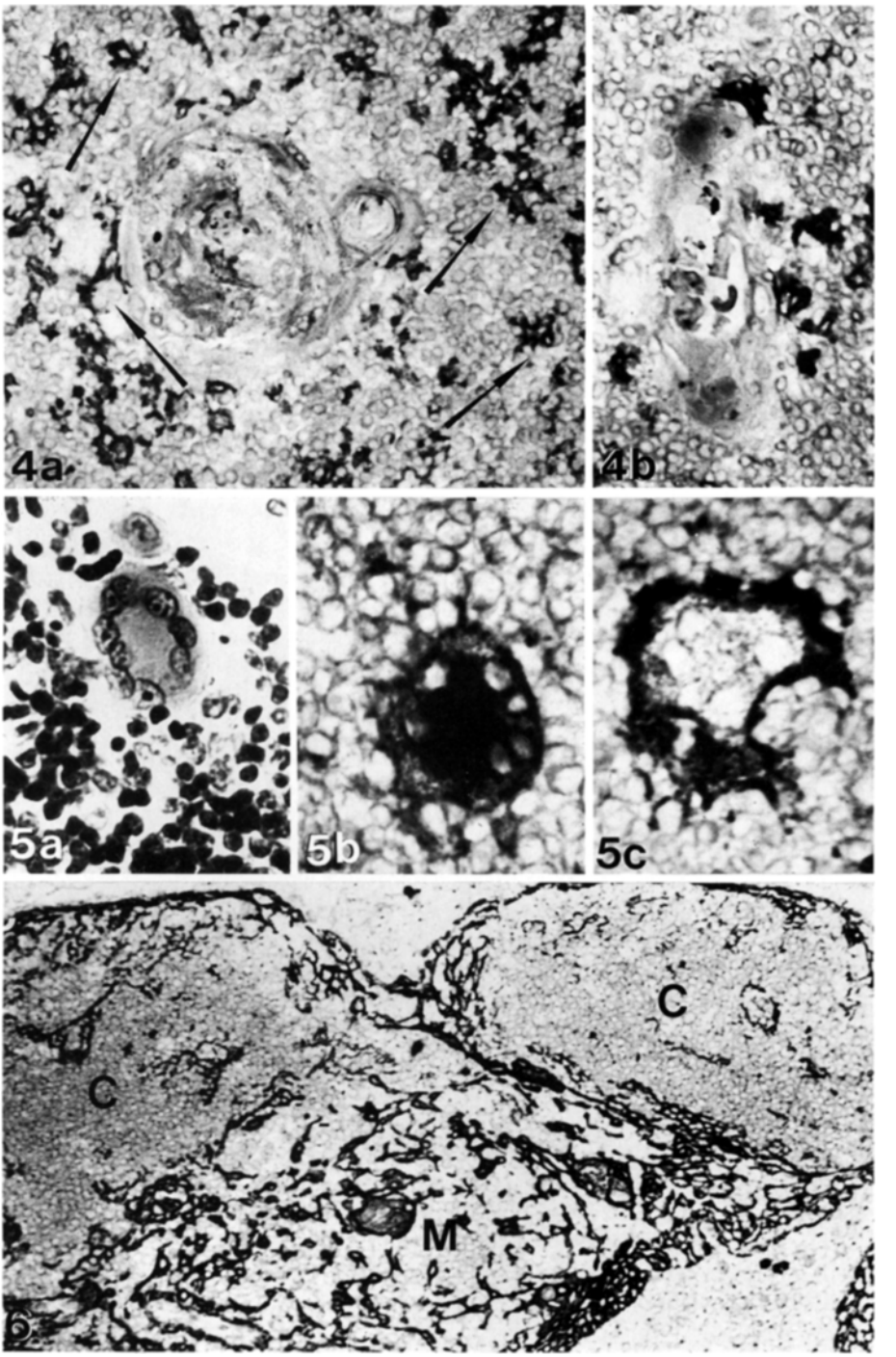

Fig. 6. Large epithelium-free cortical (*C*) foci in the thymus in MG demonstrated by absent immunoreactivity with antibody 35βH11 against keratins. Note also the large-meshed splitting of the medullary (*M*) epithelium. × 190

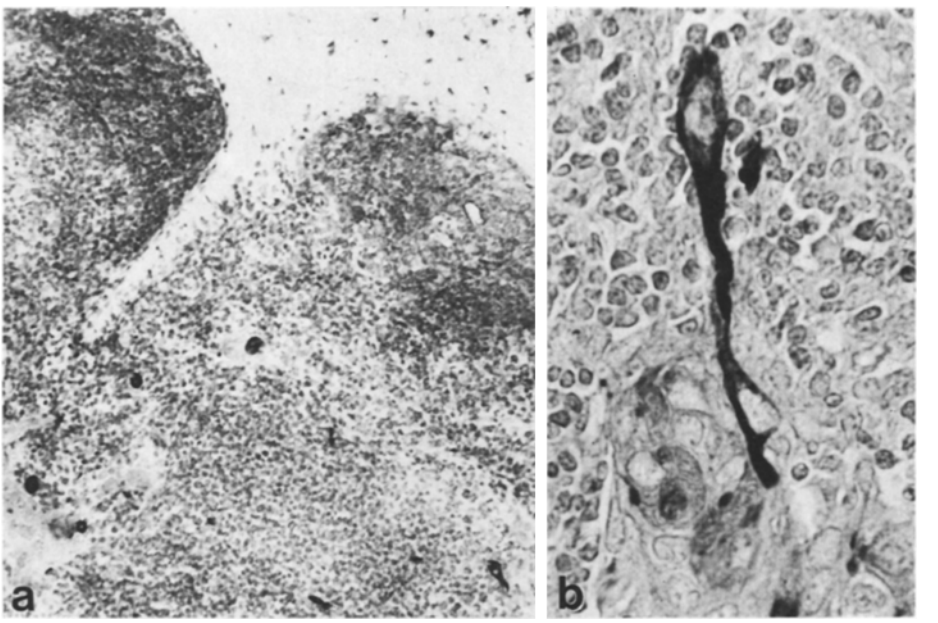

Fig. 7a–b. Thymic myoid cells in MG. **a** Immunostaining of some thymic myoid cells in the medulla by a monoclonal anti-desmin antibody on frozen section. × 150. **b** Demonstration of an elongated thymic myoid cell with contact to a HC by a polyclonal anti-desmin antibody on paraffin section. × 600

Ki-M3, HLA-DR and HLA-A, B and C, usually in a reticular pattern. Even in the normal thymus a few epithelium-free cortical foci occur. These can be defined by their negative reaction with the antibodies 35βH11 and Ki-M3 and by the depleted HLA-DR and HLA-A, B, and C pattern characterized by only variable weak staining of cortical lymphocytes.

Independent of the type of thymic alteration we found such epithelium-free cortical foci in 15 of 16 thymuses from patients with MG examined for these structures (Fig. 6). The extension of the foci was variable, ranging from few dispersed foci (eight cases) or wider areas (six cases) to large predominant zones (one case) in the cortex.

Thymic myoid cells in MG

Thymic myoid cells can be demonstrated immunohistochemically in frozen or paraffin sections of human thymus using anti-desmin antibodies. By this method myoid cells were identified in 16 of 19 cases investigated (Fig. 7a and 7b). Myoid cells have a round or elongated shape; they are scattered unevenly in the medulla, sometimes concentrated around HCs and connected to the epithelial cells within these structures. Myoid cells are not detected within lymphoid follicles.

Table 3 shows the frequency of myoid cells in relation to the type of thymic alteration in MG. The thymus contains apparently more myoid

Table 3. Frequency of thymic myoid cells and type of thymic histology in 19 cases of MG

Frequency of thymic myoid cells	Thymitis with lymphoid follicular hyperplasia ($n=10$)	Thymitis with diffuse B-cell infiltration ($n=5$)	Thymic atrophy ($n=4$)
Numerous	3	0	0
Some	4	3	2
Few	3	1	0
None	0	1	2

cells in thymitis with lymphoid follicular hyperplasia than in thymitis with diffuse B-cell infiltration. The smallest number of myoid cells is found in thymic atrophy.

Discussion

Since the first description by Sloan (1943) intrathymic GCs have been considered the most important non-neoplastic thymic change occurring in MG. Castleman and Norris (1949) called this finding lymphoid follicular hyperplasia of thymus, whereas Goldstein (1966) proposed the term 'thymitis', because he equated thymic GC formation to the changes in Hashimoto's thyroiditis and interpreted this alteration as chronic inflammation caused by autoimmune reactivity.

GCs in the thymus are not specific to MG and may also occur in other diseases, for example Basedow's disease, Hashimoto's thyroiditis, systemic lupus erythematosus and rheumatoid arthritis (Otto 1984). Even in subjects without systemic disease, a few intrathymic GCs are seen in 13% (Steinmann 1986). In the absence of thymoma, intrathymic GCs are found in 65 to 80% of patients with MG by routine stains (Alpert et al. 1971; Levine and Rosai 1978; Palestro et al. 1983), whilst in 16 to 25% the thymus appears histologically normal (Levine and Rosai 1978).

Such differences in thymic histology may be correlated with the known disease heterogeneity in MG. According to Compston et al. (1980) distinct GC formation is seen in patients under the age of 40 years, who are predominantly females, whereas thymic involution is more common in male patients over the age of 40 years.

Using immunohistological methods we have distinguished three patterns of thymic histology in MG. The first, 'thymitis with lymphoid follicular hyperplasia', is identical to lymphoid follicular hyperplasia described by other authors (Castleman and Norris 1949; Levine and Rosai 1978). The second, 'thymitis with diffuse B-cell infiltration', may correspond with 'thymus persistence' described in previous investigations of MG (Pizzighella et al. 1982). The third appearance noted was 'thymic atrophy'. The three histological patterns are associated with different age and sex distributions, as also found by Compston et al. (1980). Thymitis occurred particularly in younger females. Thymic atrophy was found in a group of older patients comprising three men and only one woman. There were some indications

that the clinical findings differed in the three types, but the small number of patients studied and the short follow-up after thymectomy does not yet allow precise clinico-pathological correlation.

A constant immunohistological feature of the thymus in MG is a pronounced B-cell infiltration of the PVS and medulla. Marked diffuse B-cell infiltrates of the thymic medulla in conjunction with GCs have already been reported by Palestro et al. (1983). We demonstrated immunohistochemically a pronounced B-cell infiltration of the medulla, even if GCs were absent or a few tiny lymphoid follicles were seen only by immunostaining of some dendritic reticulum cells with the antibody Ki-M4. This underlines the opinion of Bofill et al. (1985) that intrathymic GCs are a secondary phenomenon in response to a local polyclonal stimulation.

According to other investigations the normal thymus usually contains plasma cells and B-cells restricted predominantly to the PVS (Levine and Rosai 1978; Palestro et al. 1983). We have also observed B-cells in the normal thymic medulla (unpublished observations) and the formation of intrathymic GCs is well documented in at least 13% of subjects without MG (Steinmann et al. 1985). The significance of these observations is unclear. Since a B-cell infiltration of the thymic medulla and even intrathymic GC formation is not specific to MG, it is possible, that thymic changes similar to those in MG may occur during certain immune or autoimmune reactions of unknown cause. The extent of medullary B-cell infiltration in our MG cases was higher, or at least equivalent to the most pronounced findings in non-myasthenic controls.

The cytological and immunohistochemical identity of intrathymic and peripheral GCs has been noted by other investigators (Wiersbowsky-Schmeel et al. 1984; Palestro et al. 1983; Bofill et al. 1985). However there are differing reports of the exact localization of intrathymic GCs in the PVS (Tamaoki et al. 1971; Levine and Rosai 1978) and in the medulla (Scadding et al. 1981; Thomas et al. 1982; Palestro et al. 1983; Tridente 1985; Pizzighella et al. 1983). Our findings indicate that in MG, thymic B-cell accumulation and GC formation starts in the PVS and is followed by B-cell infiltration and the appearance of GCs in the medulla. This continuous process may be facilitated by interruptions in the boundaries between the enlarged PVS and the partly condensed medulla, which lead to fusion of the two compartments.

In fact, the structural disturbance of the PVS and medulla seems to be the crucial change in the thymic microenvironment in MG (Wekerle and Müller-Hermelink 1986). Previously Bofill et al. (1985) observed that in the thymus in MG, laminine-positive boundaries between lymphnode-like transformed areas and the medullary thymic cords break up and a new composite microenvironment results. Palestro et al. (1983) and Scadding et al. (1981) described a conspicious increase in reticulin fibres in the thymic medulla of MG patients. Söderstrom (1970) drew attention to intrathymic GCs with high endothelium venules and discussed the possibility of complete transformation of the thymus to a lymph node-like structure.

All these structural alterations permit a close interaction between medullary T-cells and the infiltrating peripheral B-cells. Immune stimulation causes GC formation in many, but not in all cases, so that thymitis without GCs can occur in MG. Regarding the clinical data, it should be emphazised that all three patients in our study receiving preoperative azathioprine treatment, showed thymitis with diffuse B-cell infiltration. It is therefore possible that immunosuppressive drugs may reduce or prevent intrathymic GC formation in MG. Since the number of GCs varies in cases of thymitis with lymphoid follicular hyperplasia, and tiny lymphoid follicles are seen in thymitis with diffuse B-cell infiltration by immunohistochemistry, overlaps between the two types of thymitis are quite possible. We have distinguished thymitis from thymic atrophy because in the latter there are only minor widenings of the PVS and little epithelial disruption at the medullary boundary. Based on the cases investigated we cannot evaluate whether overlapping between thymitis and thymic atrophy occurs. However, the relatively short duration of clinical symptoms in cases of MG with thymic atrophy make it unlikely that this change represents an end-stage of previous thymitis.

The increase in intramedullary IDCs with variable expression of the T-6-antigen (Leu 6-, and OKT 6-positive) in all three histological types may indicate activity of these antigen-presenting cells for T-lymphocytes as in the peripheral lymphatic system. The normal thymus usually contains only T-6-negative IDCs (Wekerle and Müller-Hermelink 1986). Increased numbers of IDCs have been observed by other investigators in the thymus in MG (Bofill et al. 1985; Thomas et al. 1982), although Kornstein et al. (1984) found IDC distribution to be inconspicuous. Ultrastructurally, intrathymic IDCs in MG may contain Birbeck granules, which are not seen in the normal human thymus (Wiersbowsky-Schmeel et al. 1984). T-6 antigen expression and Birbeck granules are characteristic features of epidermal Langerhans cells. We found the T-6 antigen particularly on IDCs aggregated around HCs. This suggests that there are thymic epithelium-associated IDC-like cells in MG which resemble epidermal Langerhans cells.

The significance of large intrathymic multinucleate cells at the corticomedullary junction in MG remains to be clarified. According to their immunoreactivity (positive for Ki-M1 and Ki-M6, negative for anti-keratin antibodies), these large cells belong to the macrophage system. It is remarkable that they occurred in nine of 16 thymuses showing thymitis, but in none of those showing thymic atrophy.

A similar thymic expression of HLA-DR antigens in MG and in controls was recorded by Kornstein et al. (1984). In our MG cases there were occasional thymic medullary foci exhibiting a weaker or absent HLA-DR expression in epithelial cells and lymphocytes. However we did not see the total absence of HLA-DR expression in the medullary epithelium observed by Bofill et al. (1985).

In spite of the thymic structural disturbance and B-cell infiltration in MG, the distribution of T-lymphocyte differentiation antigens did not differ conspiciously from that in the normal thymus. This observation accords

with the results of other investigations (Thomas et al. 1982; Tridente 1985; Kornstein et al. 1984).

Since cortical epithelial differentiation quite often predominates in MG-associated thymomas (Marino and Müller-Hermelink 1985; Müller-Hermelink et al. 1985; Müller-Hermelink et al. 1986), it needs to be emphazised that the structural integrity of the cortex is quite well preserved in the thymus in MG in the absence of thymoma. Some investigators have the impression that lymphoid follicular hyperplasia causes a compression and atrophy of the cortex (Levine and Rosai 1978; Pizzighella et al. 1983), whereas Bofill et al. (1985) observed no pathologic cortical involution.

Until now little attention has been paid to epithelium-free cortical foci (35βH11-, and Ki-M3-negative), which contain immature thymocytes (Leu 6/OKT 6-positive, weakly HLA-DR- and HLA-A, B and C-positive) which have no contact with 'educating' epithelial cells. These foci have been described in the normal thymus (Müller-Hermelink et al. 1982). We identified thymic epithelium-free cortical foci in 15 of 16 cases of MG and gained the impression that they are more extended in MG than in the normal thymus. Further studies are needed to show whether this phenomenon indicates degeneration of the cortex or if uncontrolled T-lymphocyte clones lacking self-tolerance arise from such foci.

Our results confirm that in the thymus in MG, B- and T-cell interaction and immune stimulation occur as in the peripheral lymphatic system. AchR-reactive T-cells and B-cells producing anti-AchR antibodies have been found in thymic cell suspensions in MG and indicate the occurence of an autoimmune process (Scadding et al. 1981; Wilcox et al. 1983; Melms et al. 1986). The incriminated stimulating antigen is apparently expressed by thymic myoid cells (Kao and Drachman 1977; Wekerle et al. 1978) and is probably presented to the T- and B-cells by activated IDCs and dendritic reticulum cells. According to our recent experience monoclonal anti-AchR antibodies facilitate the immunohistochemical staining of receptor-bearing thymic myoid cells in frozen sections (Melms et al. 1986). In the present study we investigated the number and distribution of thymic myoid cells marked by anti-desmin antibodies. Myoid cells were most numerous in thymitis with lymphoid follicular hyperplasia and least numerous in thymic atrophy.

Thus the grade of intrathymic immune reaction and the histological change in the thymus in MG may depend on differing numbers of thymic myoid cells. In both normal subjects and patients with MG we have found that the average number of thymic myoid cells decreases with advancing age (Kirchner et al., unpublished work). This may explain why the two types of thymitis predominate in younger persons whereas thymic atrophy is more common in older patients with MG.

The role of the thymus in the pathogenesis of MG in old patients is not clear. The question also remains unanswered whether an intrathymic immune reaction against AchR-expressing myoid cells initiates MG or whether thymic involvement is a secondary phenomenon during an autoimmune process initiating in the peripheral lymphatic system. The differing age of onset of MG may be due to more than one mechanism causing

a breakdown in tolerance to AchR and to variable HLA genotypes rendering individuals susceptible to one or other of these mechanisms, as discussed by Compston et al. (1980). The assumption of a primary pathogenesis of MG in the thymus is still hypothetical and is supported mainly by the benefical effects of thymectomy seen most commonly in younger MG patients with intrathymic GCs, but occurring also in older MG patients without GCs (Alpert et al. 1971; Simpson 1958).

Our study underlines the view that the intrathymic GC formation and anti-AchR antibody synthesis is not the starting event in MG, but may follow an intrathymic T-cell activation (Scadding et al. 1981; Bofill et al. 1985; Wekerle and Müller-Hermelink 1986). Thus the crucial changes that may explain a primary role of thymus in MG, are possible interactions of uncontrolled T-lymphocytes, macrophages / reticulum cells and AchR-bearing myoid cells as well as thymic structural disturbances facilitating a stimulation of B-cell infiltrates by AchR-sensitized T-lymphocytes.

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