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The Thymus in Myasthenia Gravis

Changes Typical for the Human Disease Are Absent in Experimental Autoimmune Myasthenia Gravis of the Lewis Rat

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In human myasthenia gravis (MG) formation of autoantibodies against acetylcholine receptor (AChR) is commonly associated with thymic changes termed lymphofollicular hyperplasia (LFH). To learn whether the thymic lesions of human MG are primary changes in the autoimmune pathogenesis, or rather secondary events caused by peripheral autoimmunization, the authors compared the pathologic changes of MG thymuses with the thymuses of Lewis rats with experimental autoimmune myasthenia gravis (EAMG). EAMG was induced either actively by immunization with AChR, or transferred passively with monoclonal antibodies (mAb) binding to AChR. The clinical diagnosis of EAMG was confirmed by electromyography. Germinal centers, which are typical for human MG thymuses, were not detectable in the thymus of EAMG rats. Scattered B cells were seen as normal components of the thymic medulla. In EAMG their number was not augmented, nor were they accumulated focally. The perivascular spaces (PVS) were not distended and the amount of reticulin was not increased. Thymic myoid cells were identified in EAMG as well as in control thymuses; their cellular microenvironment was inconspicuous. Both in normal and in EAMG thymuses, a subpopulation of myoid cells expressed the main immunogenic region of the AChR. Heavily affected rats showed a severe cortical involution, but no specific changes of the medulla. The fact that none of the thymic lesions characteristic for human MG was found in EAMG is compatible with the concept that the thymic changes in MG are primary events in the autoimmune pathogenesis of this disease. (Am J Pathol 1991, 139:995–1008)

In myasthenia gravis (MG), motor endplates are functionally impaired by autoantibodies binding to the nicotinic AChR. The clinical consequences are the typical fatigability and muscle weakness. Several lines of evidence indicate a crucial role of the thymus in the pathogenesis of MG. First, characteristic structural aberrations are regularly found in myasthenic thymuses: 60–80% of MG thymuses show a marked lymphofollicular hyperplasia (LFH), whereas 10–15% contain an epithelial tumor.[1] The thymus of both healthy individuals and MG patients regularly harbors AChR-expressing myoid cells.[2,3] AChR or a related protein may be expressed by thymic epithelial cells.[2,4] In the thymus, the main immunogenic region of the AChR, however, has been detected only on myoid cells.[2] Antibodies from MG patients react to both striated muscle and thymus.[5]

Then, the changes of MG thymuses point to immunopathologic events. In contrast to normal organs, they often contain cell clusters composed of myoid cells, interdigitating cells, and $CD3^+4^+$ T lymphocytes.[6] The thymus of MG patients commonly harbors AChR specific $CD4^+$ T cells.[7] Furthermore, in MG thymuses AChR-reactive B cells have been demonstrated,[8] which seem to be in an activated state.[9] Finally, thymectomy is an established beneficial therapy for MG, especially when applied in early stages of the disease.[10]

The observation that thymic stroma cells can be induced to differentiate to "classical" striated muscle cells,[11] expressing nicotinic AChR,[12,13] led to the hypothesis that in MG the autoimmune pathogenesis is prompted within the thymus by an aberrant confrontation of myoid cells with AChR-specific autoimmune T lymphocytes.[14] This concept postulates that the activated thymic autoimmune T cells emigrate to the peripheral immune system, where they induce complementary B lymphocytes to produce myasthenogenic autoantibodies.

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To verify whether the thymic lesions in MG are primary events, rather than secondary sequels of an extra-thymic anti-AChR autoimmune response, we examined the thymus in experimental autoimmune myasthenia gravis (EAMG) of the Lewis rat.

In EAMG, the pathogenesis is induced either by active immunization with AChR[15,16] or by passive transfer of antibodies to AChR.[17–19]

EAMG thus does not depend on primary events within the thymus. MG-like lesions in EAMG thymuses would argue in favor of their secondary nature. None of the characteristics of human MG thymuses were noted in EAMG, which may support an active role of the thymus in the pathogenesis of human MG.

Material and Methods

Preparation of AChR

Nicotinic AChR was purified from *Torpedo californica* (Pacific Biomarines Laboratories, Venice, CA)[20] and dialyzed extensively against 0,005 M phosphate buffer to remove residual detergent.

Animals and Rating of EAMG

Female Lewis rats (Charles River breeding facilities, Sulzfeld, FRG) aged 8–10 weeks were used in our experiments. Disease intensity was graded as follows: 1) weak grip with fatigability, 2) hunched posture with lowered head, and 3) tremor, no grip, moribund.[16]

Actively Induced EAMG

The animals were immunized with 50 μg AChR emulsified in complete Freund's adjuvant (CFA). A volume of 0.3 ml was distributed into the hind footpads and two sites on the back. As additional adjuvant, 0.5×10^{10} *Bordetella pertussis* organisms (Gutenberg-Apotheke Bern, Switzerland) were injected into the dorsum of each hind foot. Two weeks later, the animals received again 50 μg AChR/CFA on multiple sites of the back. As controls, we used four untreated rats, and five rats that had received ovalbumin/CFA/*B pertussis*.

Passive Transfer of EAMG

We used the mAb 35, an Ab directed against the main immunogenic region of the AchR. Control rats received the mAb 118, directed against the cytoplasmic part of the AChR. Both antibodies are of the rat IgG1 isotype[19,21] and such antibodies activate complement.[22] Culture supernatant of the hybridoma cells was precipitated with 45% ammonium sulphate and subsequently dialyzed against phosphate-buffered saline. This resulted in a 40-fold concentration of the antibody. The animals received 1.5 ml of this precipitate IP.

Electromyography

The rats were anaesthetized by subcutaneous injection of Hypnorm (Fentanyldihydrogen-citrate; 1 ml/kilogram body weight). The plexus brachialis was supramaximally stimulated with percutaneous needle electrodes at rates of 3 and 10 Hz. Evoked muscle compound action potential was measured with superficial electrodes from the lower forelimb (Disa Electronic Type 14E11, Herley, UK; Oscilloscope 5103, Tetronix Inc., Beaverton, OR). Amplitude of a single evoked potential was determined and decrements of the serial stimulation were calculated as the difference between the first and the fifth amplitude divided by the initial amplitude and considered significant if higher than 10%.[23]

Rats with actively induced, chronic EAMG were challenged with curare.[23] These animals received 0.2 μg dimethyl-curarin HAF, control rats 0.8 μg by IP injection in saline solution. Muscle responses were measured every 5 minutes postinjection for 1 hour.

Purification of Monoclonal Abs and Conjugation with Biotin

Culture supernatants of hybridomas 35 and 9–8 were precipitated with a 45% saturated ammonium sulphate solution and further purified on a diethylaminoethyl cellulose (DEAE) column. The antibodies were then conjugated with biotin as described elsewhere.[24] The capacity of the biotinylated antibodies to bind antigen and avidin was examined in an ELISA test. At a dilution of 1:10,000, the reaction of the conjugated mAb was clearly above background (data not shown).

Preparation of the Tissues for Morphologic Examination

Animals were anaesthetized with ether and subsequently exsanguinated by heart puncture. Some specimens were fixed with 4% paraformaldehyde overnight for paraffin embedding and others were snapfrozen in isopen-

tan cooled by liquid nitrogen and stored at −80°C until use.

Routine Histology

Paraffin sections (5 μm thick) were stained with hematoxylin and eosin (H&E) and silver stains.

Immunohistochemistry

All immunohistochemical reactions were performed at room temperature on cryostat sections (6 μm) that were air dried for 3 hours, fixed with freshly prepared paraformaldehyde for 4 minutes, and then washed with tris-hydroxy-methyl-aminomethane (TRIS) buffer (pH 7.4). mAbs used in this study are listed in Table 1.

Single Staining with Peroxidase

Depending on the primary antibody three different staining protocols (a, b, c) were used that can be described in the following flow charts:

a) phase 1: fetal-calf serum (FCS), phase 2: mouse mAb, phase 3: biotin sheep anti-mouse Ig (Amersham; 1:200), phase 4: avidin-peroxidase (Sigma; 1:200)

b) phase 1: FCS, phase 2: anti-troponin mAb, phase 3: rabbit anti-mouse Ig peroxidase (Amersham; 1:50), phase 4: donkey anti-rabbit Ig-peroxidase (Amersham; 1:50)

c) phase 1: 30% rat serum, phase 2: biotin rat mAb, phase 3: avidin-peroxidase.

The mAb 35 was diluted in 30% rat serum, the other antibodies were diluted in TRIS buffer containing 10% FCS. Avidin-peroxidase was diluted in TRIS buffer containing 1% bovine serum albumin and 0.5 M NaCl to reduce unspecific binding of avidin. Anti-mouse Ig was absorbed with rat serum and endogenous peroxidase was blocked by incubating the sections after the primary antibody in methanol containing 0.5% H_2O_2 for 20 minutes. The slides were washed with TRIS buffer after each step. Finally, they were incubated with 0.05% diaminobenzidine for 10 minutes and counterstained with hemalum.

Single Staining with Alkaline Phosphatase

Alkaline phosphatase stainings were done with the avidin/biotin system as described earlier, but using avidin-alkaline phosphatase (Sigma), naphtol-AS-MX-phosphate as substrate and fast blue BB salt (Sigma) as coupler. The substrate was dissolved in N,N-dimethylformamide (10 mg/ml) and then diluted 1:50 with 0.1 M TRIS/HCl (pH 8.2). Endogenous alkaline phosphatase was inhibited by 1 mM Levamisol. Immediately before use, the coupler was added (1 mg/ml) and the mixture was filtered. The slides were incubated for 10 minutes. The reaction was stopped with TRIS buffer, and no counterstaining was done.

Immunohistochemical Double Staining

To characterize the microenvironment of thymic myoid cells, troponin was stained as described earlier resulting in a brown-colored staining. After finishing the peroxidase reaction and washing the slides with TRIS buffer, ED1 or ED2 were demonstrated with alkaline phosphatase in a blue color. No counterstaining was done.

Table 1. *Primary Antibodies Used for Immunostaining*

Antibody	Dilution	Species Ig class	Epitopes or cells labeled	Source
KiB1R	1:300	Mouse IgG1	All types of B cells	Dr. Wacker
KiM4R	1:1	Mouse IgG1	Follicular dendritic cells	Dr. Wacker et al[30]
ED1	1:100	Mouse IgG1	Monocytes Dendritic cells Macrophages	Camon Wiesbaden, FRG
ED2	1:100	Mouse IgG2a	Macrophages	Camon
W3/13	1:100	Mouse IgG1	Thymocytes, T cells Polymorphs	Camon
Ox6	1:500	Mouse IgG1	MHC class II	Camon
Ox19	1:100	Mouse IgG1	Thymocytes, T cells	Camon
Ox39	1:100	Mouse IgG1	IL-2 receptor	Camon
Antitroponin	1:300	Mouse IgG1	Skeletal muscle	Amersham Braunschweig, FRG
35	10 μg/ml	Rat IgG1	Main immunogenic region of the AChR	Dr. Tzartos et al[19]
9-8	10 μg/ml	Rat IgG1	Myelin basic protein	Dr. Neumann[26]

Enzymehistochemical Demonstration of Cholinesterase

Sections were prepared as for immunohistochemistry and motor endplates were stained by demonstrating the activity of acetylcholine esterase.[25]

Combination of Enzyme and Immunohistochemistry

With this technique, the phenotype of the cells infiltrating the motor endplates was determined. In the first step, acetylcholine esterase was demonstrated as described earlier. After washing the immunostaining was done using alkaline phosphatase.

Controls for Immunostaining

As a positive control for KiM4R and KiB1R, we used a lymph node, for troponin, mAb 35, and mAb 9-8 tongue muscle. In the negative control, the primary antibody was replaced by 10% FCS. For evaluation of the staining with mAb 35 this mAb was replaced by mAb 9-8 in a second control section. The mAb 9-8 has the same isotype as the mAb 35 and was biotinylated in the same way.[26]

For double staining, comparative examinations on parallel sections were done with single staining, and they showed that the number of positive cells was unaffected through the double-staining procedure.

Results

Active Induction of EAMG

Among the 16 Lewis rats immunized with AChR, one animal had acute EAMG 8 days postinjection and showed the "classical" electrophysiologic signs of acute myasthenia[27] with 17% electromyogram decrement in the absence of curare treatment, and a reduction of the amplitude to 20%.

In 13 rats, typical clinical signs of chronic EAMG were seen within 30–60 days postinjection. The course of the disease was progressive and led to a moribund state 4–12 days after the onset of symptoms. The animals were weighed three times a week; severely afflicted animals lost up to 30% of their body weight. Individual rats were sacrificed at different phases of the disease, which allowed examination of thymuses from rats with mild, intermediate, and severe EAMG (Table 2). In contrast, the control animals, immunized with ovalbumin/CFA/B pertussis, or untreated, did not show signs of weakness and their weight gradually increased during the observation period.

Eight of the 13 rats with actively induced chronic EAMG were examined with EMG, and challenged with curare.[23] All of them showed a decrement higher than 20% (Figure 1). The amplitude of a single evoked potential was not reduced in these rats. The two immunized animals that did not have EAMG did not show a decrement. All control rats received the fourfold dose of curare, and yet none of them showed a significant decrement.

Passive Transfer of EAMG

Six rats were injected with mAb 35. They all developed severe EAMG (score + +/+ + +) between days 2 and 3, but the symptoms subsided almost completely by day 6. Three of these animals were killed at the peak, and the others towards the end of the disease period. Six control rats received mAb 118 and none of them became sick (Table 2).

When examined on day 2 postinjection, the rats with passive EAMG showed a significant decrement (40% ± 18%) without challenge with curare. The amplitude of the muscle compound action potential was reduced by 60% ± 25%.

Gross Architecture of EAMG Thymuses

H&E staining demonstrated a grossly unaltered medulla (Figure 2). In particular, no lymph follicles were detected in any of the thymuses examined.

Reticulin, as visualized by silver staining[28] was found in the interlobular space on the outside of each lobule

Table 2. *Rats Used for Morphologic Examination*

Disease	Number	Weight of the thymus (g)
Actively induced chronic EAMG		
+/+ +	8	0.24 ± 0.06
+ + +	4	0.06 ± 0.02
Actively induced acute EAMG + +	1	0.22
Passive EAMG on day 2–3 + +/+ + +		
Day 2 pi	3	0.14 ± 0.06
Day 6 pi	3	0.05 ± 0.01
Controls	15	0.30 ± 0.05

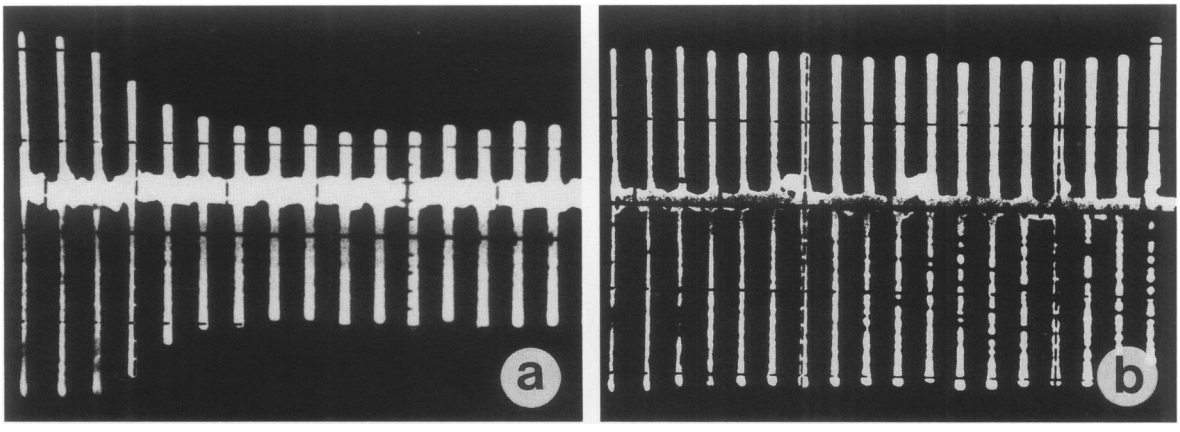

Figure 1. *Electromyography: Serial stimulation of a lower forelimb muscle.* **a:** *A rat with chronic EAMG shows a decrement after application of 0.2 μg dimethyl-curarin.* **b:** *The control animal shows no decrease of the amplitude even after challenge with 0.8 μg dimethyl-curarin.*

from where it entered to a variable degree into the cortex. Most of the medullary reticulin formed concentric rings around blood vessels, surrounding the medullary PVS. The size of the PVS, and thus the number of entrapped lymphocytes, varied between individuals, but most important, there were clearly no signs of distension or disruption of the PVS in EAMG rats. Additionally, the thymus in EAMG did not show an increased amount of reticulin fibers (Figure 2).

Animals with rapid-developing severe disease had macroscopically shrunk thymuses (Table 2). Histologic examination of these organs showed the preferential involution of the cortex (Figure 2b), with "starry sky" macrophages (Figure 2a), reflecting an abnormally high rate of cell death.

The PVS in involuted thymuses was reduced, depleted of lymphocytes, and with reticulin fibers closely attached to the blood vessels. Perilobular reticulin fibers were thickened, probably due to the shrinking of the organ (Figure 3d).

The adjuvant control thymuses did not show any changes 6–8 weeks after immunization. Their weight was the same as that of the thymus of untreated rats and histologic examination did not reveal evidence for thymic involution.

Thymic Myoid Cells

Using an antibody to troponin T reacting with striated muscle, myoid cells were found in each thymus examined. These cells were located in the medulla, often close to the cortico-medullary junction, and sometimes arranged in clusterlike aggregations.

Size and number of rat myoid cells were remarkably variable within individual thymuses. Most commonly they were round, and in some rare cases we saw elongated cells of enormous size (Figure 4a-c).

The membranes of some scattered medullary stromal cells were stained by the myasthenogenic mAb 35. Troponin$^+$ and AChR$^+$ medullary cells showed the same pattern of localization, suggesting that a minority of thymic myoid cells expresses the main immunogenic region of AChR on the surface. The pattern of distribution and frequency of cells labeled with troponin and mAb 35 were unchanged in EAMG (Figure 4d).

The Microenvironment of Thymic Myoid Cells

To characterize the cellular microenvironment of myoid cells in EAMG and in normal thymuses, we performed double staining, combining anti-troponin antibodies with the anti-phagocyte mAb ED1 (Figure 5a) in actively induced EAMG. In passive transfer EAMG, we used both mAbs ED1 and ED2 (Figure 5b).

These combinations were chosen for two reasons. First, in human MG thymuses, myoid cells are intimately associated with interdigitating cells.[6] ED1 stains rat-interdigitating cells, along with a subpopulation of macrophages.[29] Second, ED1$^+$ and ED2$^+$ mononuclear cells form the main contingent of infiltrating cells around the motor endplate in passive EAMG (Figure 7). AChR expressing myoid cells might well be attacked by the same cells.

In EAMG and in control animals, the distribution of ED1$^+$ and ED2$^+$ cells was indistinguishable. ED1 labeled cells in the cortex and medulla and especially of the corticomedullary junction. In contrast, ED2$^+$ cells were exclusively localized within the cortex.

Myoid cells were not conspicuously associated with ED1$^+$ cells (Figure 5a) in actively induced or in passively

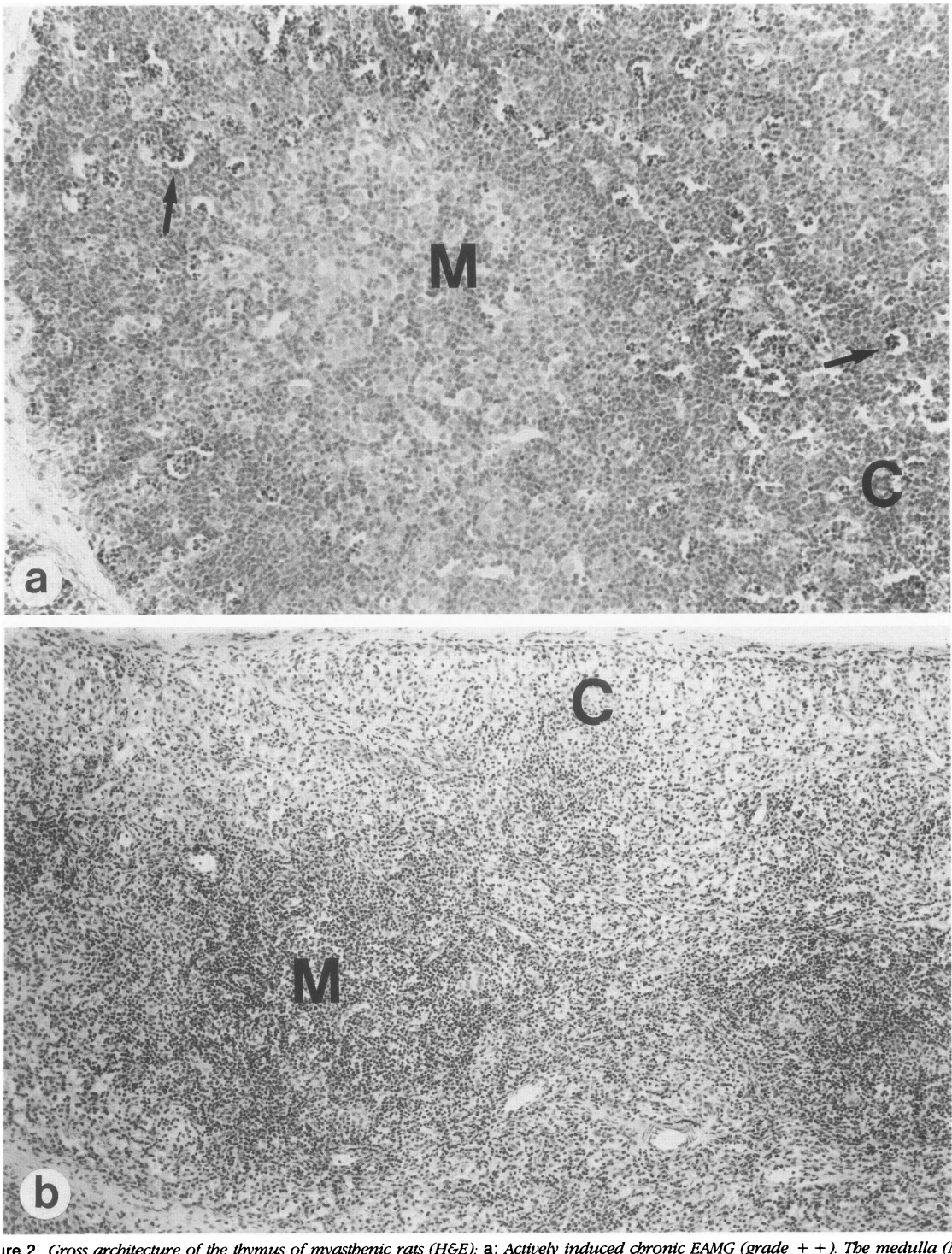

Figure 2. *Gross architecture of the thymus of myasthenic rats (H&E):* **a:** *Actively induced chronic EAMG (grade + +). The medulla (M) is normal, especially no lymph follicles are detectable. The cortex (C) shows numerous starry sky macrophages (arrow) (×220).* **b:** *Passive transfer EAMG on day 6 pi (severity grade + + + on day 3). A severe cortical involution with a higher density of cells in the medulla (M) than in the cortex (C) is evident (×105).*

transferred EAMG. There were some random contacts between interdigitating cells and myoid cells due to the high density of $ED1^+$ cells within the medullary tissue, but this was seen both in control and in myasthenic animals at similar frequency.

Even in passively transferred EAMG with its phagocyte infiltrates around motor endplates (Figure 7), the $ED2^+$ thymic macrophages remained restricted to their cortical location and did not move towards the myoid cells in the adjacent medulla (Figure 5b).

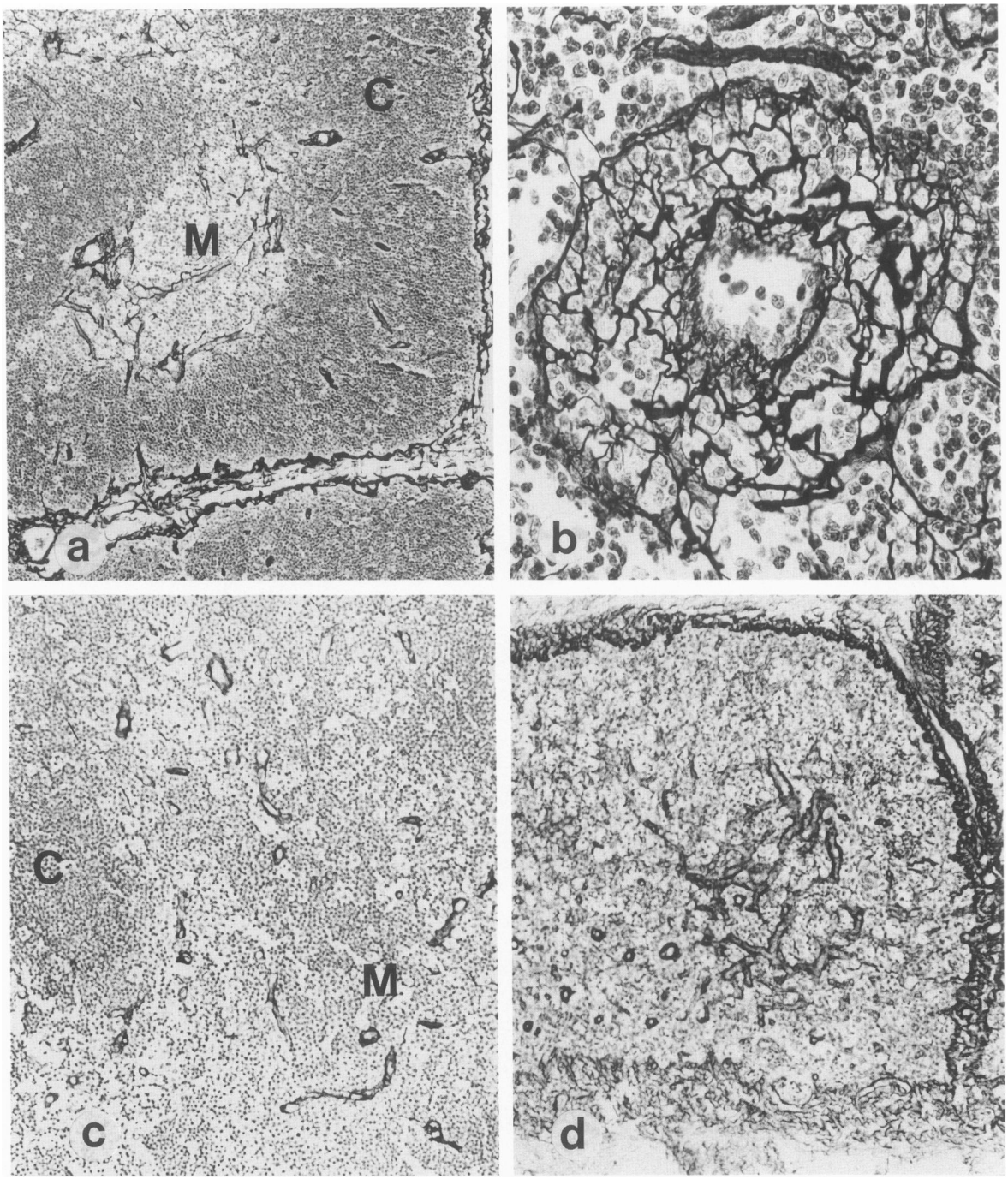

Figure 3. *Demonstration of reticulin fibres and identification of the PVS in control* (**a, b**) *and myasthenic animals* (**c, d**) *with silver staining. The reticulin fibers surround each lobule and enter the cortex to a variable degree. Most of them are found around the blood vessels* (**a,** *×120), these fibers form with concentric like rings the PVS* (**b,** *×470). Neither in mild* (**c,** *×240), nor in severe* (**d,** *×110) chronic EAMG is the PVS distended and the amount of reticulin fibers is not increased.* **d**: *Due to thymic involution cortex and medulla are no longer distinguishable by means of cell density. The fibers surrounding the lobules are thickened and convoluted, indicating the shrinkage of the organ.*

Lack of Germinal Centers and Diffuse B-cell Infiltration in Thymuses of EAMG Rats

The mAb KiB1R stains all types of B cells found in a normal lymph node (Figure 6d; personal communication with Dr. H-H Wacker, Institute of Pathology, University of Kiel, FRG). Scattered B lymphocytes are components of the normal rat thymus (Figure 6b). Most of these thymic B cells are located in the medulla, with a few of them in the cortex as well. Sometimes they form small groups. There is no predilection to the PVS, although they are not ex-

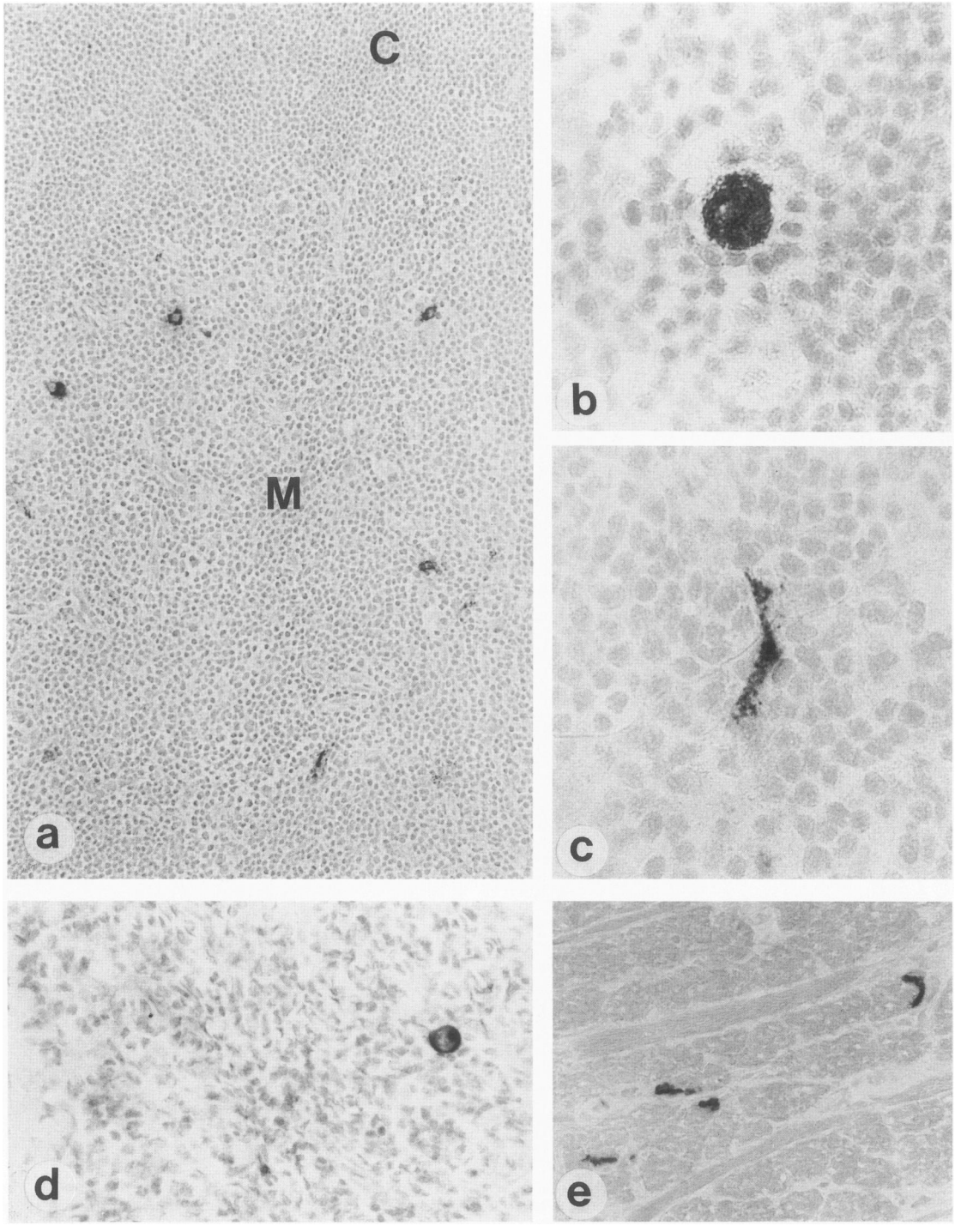

Figure 4. *Identification of thymic myoid cells (Immunoperoxidase)* **(a–c)** *with an antibody against troponin in the medulla (M) of controls* **(a,** *×210) and rats with active chronic EAMG (***b,c** *×800).* **d:** *AChR expression demonstrated with mAb 35. The surface of a myoid cell is stained. This rat had active chronic EAMG + + + and the thymus shows a severe involution. Witness the lower density of cells at the end of the lobule on the left edge of the picture (×440).* **e:** *As a control for mAb 35, motor endplates in a skeletal muscle are stained (×330).*

cluded from this compartment. In EAMG thymuses, there was no increased diffuse B-cell infiltration, nor did we see B lymphocytes in follicular aggregates (Figure 6a).

The lack of germinal centers in EAMG thymuses was corroborated by the use of mAb KiM4R,[30] which specifically stains follicular dendritic cells, the stroma cells of germinal centers (Figure 6e). This antibody did not stain any lymph follicle within EAMG thymuses (Figure 6c).

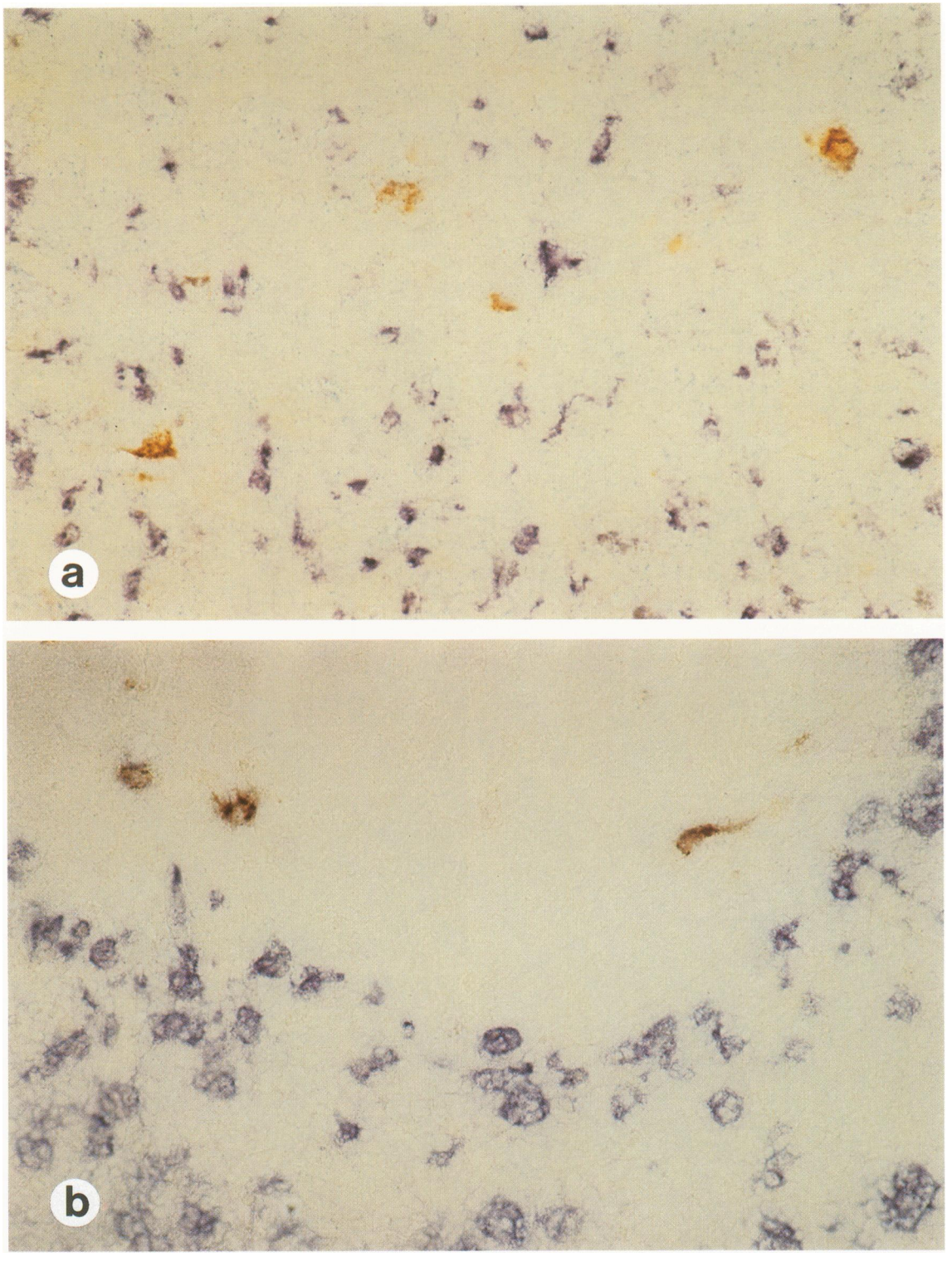

Figure 5. *Microenvironment of thymic myoid cells (double immunostaining; ×440)* **a**: *Troponin positive myoid cells (immunoperoxidase, brown) are not associated with interdigitating cells (alkaline phosphatase, blue) in active chronic EAMG.* **b**: *Passive transfer EAMG, 2 days pi ED2$^+$ macrophages (alkaline phosphatase, blue) maintain their cortical location and do not migrate towards the myoid cells (immunoperoxidase, brown) in the adjacent medulla, although they infiltrate motor endplates at the same time (Figure 7c).*

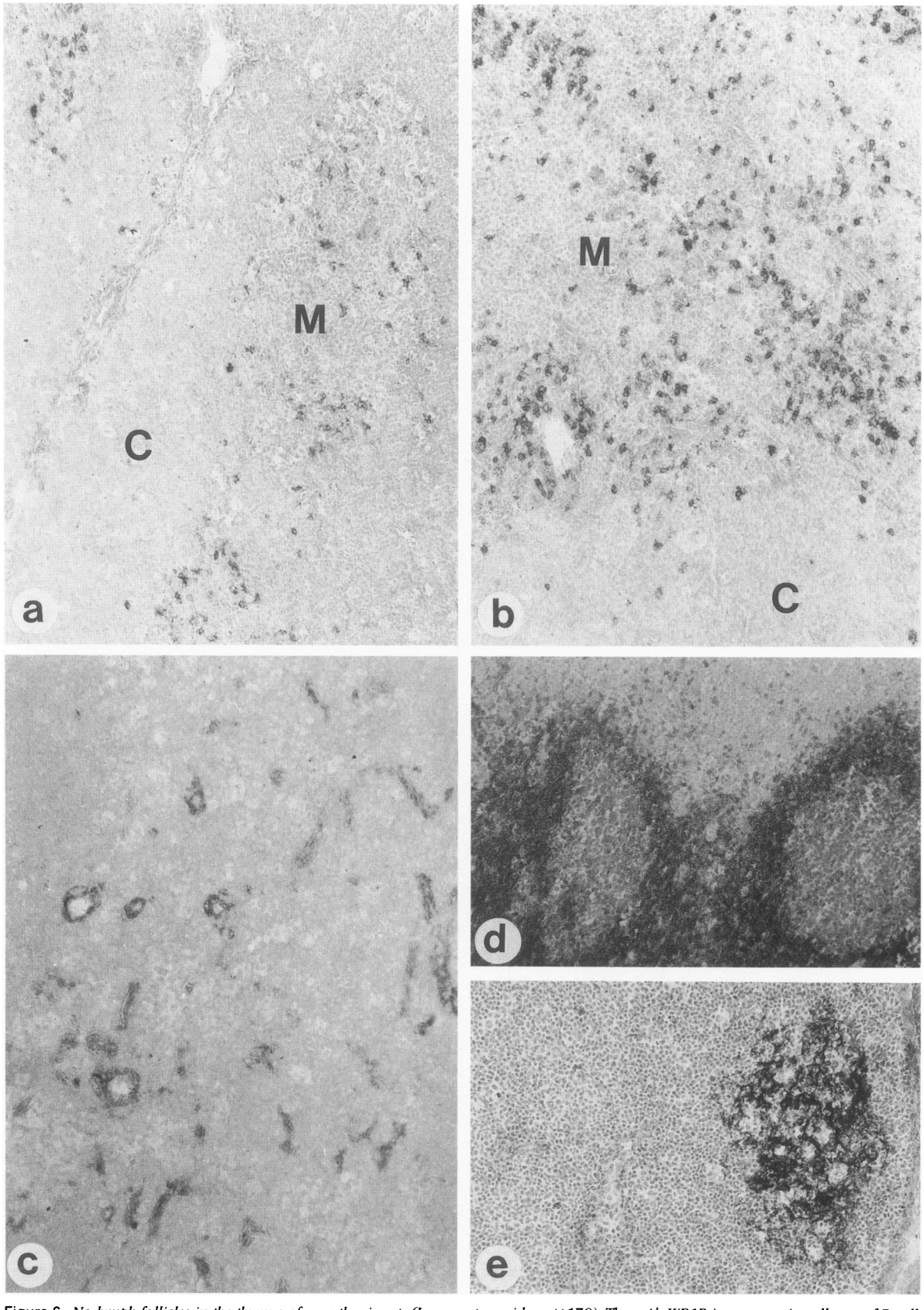

Figure 6. *No lymph follicles in the thymus of myasthenic rats (Immunoperoxidase; ×170). The mAb KiB1R immunostains all type of B cells in a lymph node* (d). *This mAb demonstrates that B cells are a normal component of the thymic medulla both in rats with active chronic EAMG* (a) *and controls* (b). *In EAMG their number is not augmented, nor are they aggregated to follicles. The mAb KiM4R binds to follicular dendritic cells and stains follicles in a lymph node* (e). *In the thymus of a rat with actively induced chronic EAMG, no lymph follicles with germinal centers are demonstrable* (c), *this mAb just shows a cross reactivity with cells lining the blood vessel.*

Lymphorrhagia of the Motor Endplate

Rats with mAb-transferred acute EAMG regularly showed infiltrations at the motor endplates (Figure 7a), which were most marked at the peak of disease.

Immunohistochemical analysis showed that the endplates were invaded by a heterogeneous population of phagocytic cells (Figure 7b, c). Infiltrating cells expressed MHC class II (OX6), were stained with ED1, to a lower extent with ED2, and few with W3/13. No cells expressing CD5 (OX19) or the interleukin 2 receptor (OX39) were detected.

Discussion

The present studies were designed to test the hypothesis of the intrathymic pathogenesis of MG. This concept postulates that the first step of the autoimmune pathogenesis of MG involves an aberrant interaction between AChR-expressing thymic myoid cells and antigen-presenting interdigitating cells on the one hand, and AChR-specific, autoimmune thymic T lymphocytes on the other.[14,31]

As experimental model, we used EAMG of the rat. We studied the pathology of the thymus in two distinct MG models. Chronic EAMG was actively induced by immunization with AChR/CFA. This model resembles human MG in its clinical, immunologic, and electrophysiologic features, as well as in the ultrastructural changes of the motor endplate.[32,33] In addition, MG can be transferred with antibodies to AChR from patient to animal,[17] from an immunized animal to a naive one,[18] or with appropriate monoclonal antibodies.[19] This model leads to phagocyte infiltrates at the motor endplate[18] and may resemble acute and early phases of human MG where lymphorrhagias have been detected occasionally.[34]

EAMG shares its autoimmune effector mechanisms with MG. The early stages of the pathogenesis are, however, fundamentally different. Obviously, EAMG is not triggered by spontaneous intrathymic cell interactions, but by an autoimmune response to AChR mediated either by immunization, or by transfer of pathogenic immunoglobulins. Hence, any thymic changes seen in EAMG would be of secondary nature.

We first focussed on LFH, which is found in the thymuses of 60–80% of MG patients, and which is especially closely associated with MG of young women, having the HLA DR3 haplotype.[35] LFH is characterized by numerous and prominent lymph follicles with germinal centers, which dilate the thymic medulla.[1]

Apart from conventional histology, germinal centers can be demonstrated immunohistochemically with antibodies to B lymphocytes and to "their" stroma cells, the follicular dendritic cells. This technique is highly sensitive,

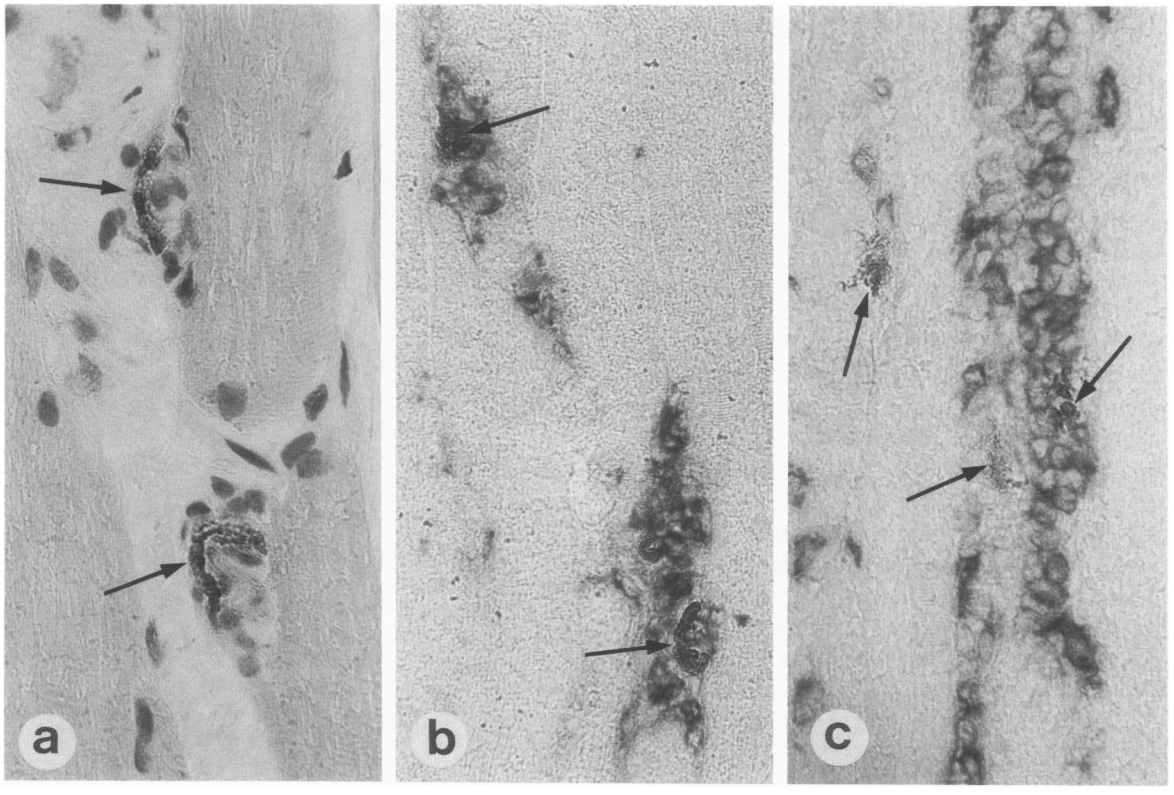

Figure 7. *Infiltrates at the motor endplates (enzyme histochemical demonstration of cholinesterase; arrow; ×420) in passive transfer EAMG.* **a:** *The infiltrating cells (haemalum) seem to separate the endplates from the underlying muscle fiber.* **b, c:** *The infiltrates (alkaline phosphatase) are marked with ED1* **(b)** *and ED2* **(c).**

allowing the identification of otherwise undetectably small or early lymph follicles.[28]

Irrespective of the technique used, no lymphoid follicles were demonstrable in the EAMG thymuses examined in our series. Scattered B cells, which are physiologic components of the thymic medulla, were seen in EAMG thymuses, but were neither augmented by number, nor aggregated to follicles.

A second hallmark of the human MG thymus is the distended PVS. In myasthenic thymuses, the PVS areas are enormously enlarged and crammed with peripheral lymph–node-like lymphoid tissue.[28] Often the reticulin fiber network is increased.[37–39] No comparable changes of the PVS or the thymic extracellular matrix were seen in EAMG using silver staining techniques.

A third key lesion in the MG thymus is a characteristic alteration of the cellular microenvironment around myoid cells. Recent morphometric analyses[6] have established that, in MG, myoid cells are intimately associated with interdigitating cells. In MG, but not in normal human thymuses, interdigitating cells seem to surround myoid cells with their dendritic processes. Using immunohistochemical double staining combining antibodies against rat interdigitating cells[29] with anti-troponin antibodies, no nonrandom association between both cell types were seen in EAMG thymuses.

These morphologic results document that neither active induction nor passive transfer of EAMG leads to thymic changes similar to those found in the human MG. Our studies suggest that in EAMG the thymus does not play an essential role as it does in human MG. This is in harmony with the observation that thymectomy of adult rats after immunization with AChR does not influence the course of subsequent chronic EAMG.[40] This is remarkable, because in human disease thymectomy is frequently beneficial.[10]

The reasons for our failure to demonstrate MG specific thymic changes in EAMG could have been trivial. For example, the interval between the myasthenogenic treatment and thymus examination could have been too short to allow formation of demonstrable germinal centers. This is not probable, as reconstitution experiments of irradiated rats indicate that germinal centers can form within 1 week.[41] In actively induced EAMG, however, we examined thymuses over 2 months postinjection.

Several other factors could account for the lack of lesions in the EAMG thymuses. First, the target autoantigen could be absent from the thymus. This is not the case, since we regularly demonstrated surface expression of AChR on myoid cells. Others demonstrated thymic AChR biochemically.[42] Secondly, the rat myoid cells could be inaccessible to AChR specific autoantibodies and/or autoimmune T lymphocytes. Again, this possibility is weakened by the fact that in rodents, both soluble proteins[43,44] and antigen specific T lymphocytes have ready access to the thymic medulla.[45,46]

The most conspicuous pathologic change observed in our EAMG thymuses was a profound, mainly cortical involution, which was noted in severely affected animals. This type of involution has been known to be related to stress situations,[47] presumably mediated by cortisone. In addition, the small and condensed PVS indicates a decrease of the thymic lymphocyte traffic.

Cortical involution is totally different from thymic atrophy found in about 20% of MG cases. In these thymuses, adipose tissue prevails, and in the scattered remaining islands of parenchyme, distribution of lymphocytes to cortex and medulla is inconspicuous.[28]

Taken together, the data reported show that in two different models of EAMG, none of the changes typical for MG thymuses were demonstrable. This observation supports, but does not formally prove, the hypothesis of a primary involvement of the thymus in the pathogenesis of MG.

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