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THE THYMUS IN MYASTHENIA GRAVIS: AN IMMUNOHISTOLOGIC STUDY

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INTRODUCTION

Abnormalities of the thymus have long been noted in patients with myasthenia gravis (MG).[1,2,3] Morphologically, the presence of germinal centers in the medulla of the MG thymus was described by Castleman in 1949.[1] Functionally, the MG thymus cells have altered immunologic activity.[2,3] Recently the development of monoclonal antibodies to T cell subsets allows for the furthur investigation of the MG thymus.[4] We have used such monoclonal antibodies to investigate the MG thymus in tissue sections. We have compared these results to findings obtained in studies of suspended cells from the same thymus specimens.

METHODS

Fresh tissue was obtained from twelve MG patients (age range 20-40) undergoing thymectomy and from three controls (ages 4/12, 33, 42 years) undergoing surgery for localized cardiac disease. Tissue sections were stained using the avidin-biotin immunoperoxidase system as described[5] with monoclonal antibodies to the following antigens: T11, T4 (Coulter), T6, T8 (Ortho), IgM, and IgD (Cappel), or with rabbit anti-keratin antibody (Dako). Appropriate controls were included in each run. In microscopic examination (using an ocular grid) of replicate sections, the percentage of cells bearing each phenotypic marker was determined for the cortical and medullary areas. At least 100 cells were counted in each area of the thymus in each section.

For electron microscopy of tissue sections, small pieces (0.5 mm) of thymic tissue from three MG patients were fixed in 2% buffered gluteraldehyde (Karnovsky's fixative) for four hours and then washed in cacodylate buffer (0.1 M pH 7.4) for three days. Tissue for immunoelectron microscopy was then incubated for three days at 4 C with either PBS or monoclonal antibody to Ia antigen (Ortho) diluted 1:50 in PBS. The antigen was then visualized using an avidin-biotin immunoperoxidase technique as described.[5] Sections were observed and photographed with a Philips 200 electron microscope at 60 KV.

For electron microscopy of cell suspensions, thymic lymphocyte suspensions were processed for immunoelectron microscopy using an avidin-biotin immunoperoxidase technique as described.[5] One MG patient and one control were studied.

RESULTS

In tissue sections of MG and control thymus, all cortical lymphocytes appeared positive for T11, T4, T6, and T8 (fig. 1). None of the cortical cells were IgM or IgD positive.

In the medulla of both MG and control thymus, approximately half of the cells were T11 positive (Table I). About one third were T8 positive, half were T4 positive, and 10% were T6 positive. We found no difference in the pattern of T cell subsets when MG was compared to control thymuses.

Germinal centers were frequent and prominent in the medulla of the thymic tissue from 10 of the 12 MG specimens. A single germinal center was seen in one of the control thymus specimens. Mantle zone lymphocytes were both IgM and IgD positive whereas cells in the germinal center were generally only IgM positive. This pattern is similar to that seen in germinal centers of lymph node sections. Apart from the germinal centers, scattered medullary cells positive for IgM and IgD were identified in the thymus of both MG and control thymuses (Table II). T11 positive cells surrounded the germinal centers with occasional T11 and T4 positive cells within the center. T8 positive cells were less frequent and T6 positive cells non-existent in the germinal center.

Localization of Ia antigen was similar for MG and control thymus (fig. 2). The staining of dendritic processes in the cortex and a confluent pattern in the medulla precluded meaningful enumeration and identification of Ia positive cells by light microscopy. In electron microscopic examination of MG thymus tissue sections, Ia antigen was localized to the surface membrane of epithelial cells and interdigitating dendritic cells. In addition, Ia positive portions of the surface membranes of lymphoid cells were noted adjacent to Ia positive non-lymphoid cells (fig.3). Electron microscopy of MG thymus cell suspensions showed occasional

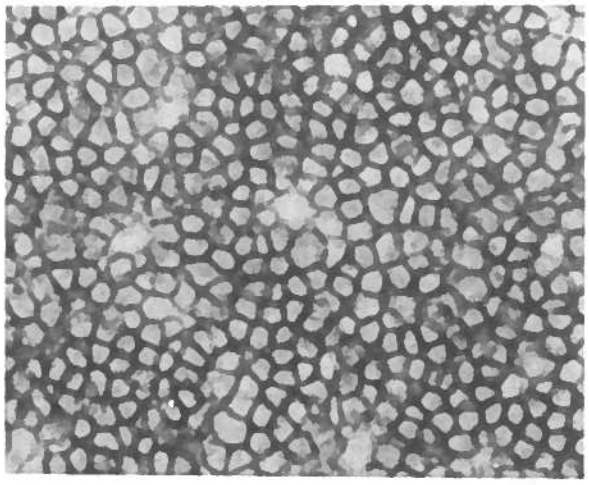

Fig. 1. MG thymic cortex examined for T11. Virtually all cortical lymphocytes are T11 positive. T11 negative cells appear to be non-lymphoic. This same pattern is seen in the cortex for T11, T4, T6, and T8. (400 x)

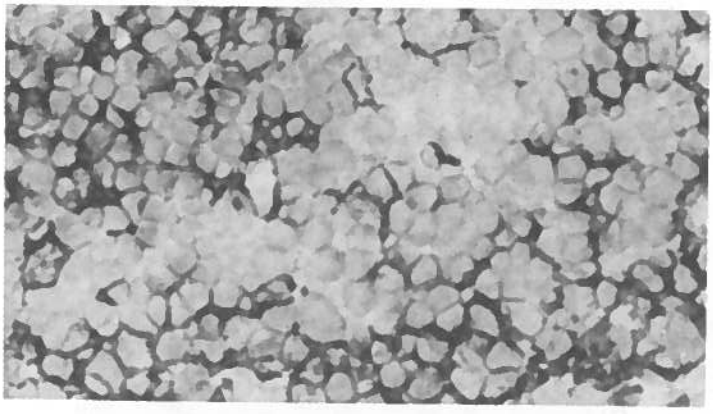

Fig. 2. Frozen section of MG thymic cortex demonstrates localization of Ia antigen. The dendritic pattern precludes enumeration of positive cells. (400 x)

lymphocytes with Ia antigen on their entire surface.

Frozen sections of thymus from 5 MG patients and 1 control were examined by immunoperoxidase technique for keratin. (fig. 4) In the cortex, keratin was localized to long dendritic processes and occasional large cells. In the medulla, numerous large cells and all Hassall's corpuscles were keratin positive. No difference in

TABLE I

T CELL SUBSETS IN THE THYMUS

TISSUE SECTION DATA (% POSITIVE CELLS)

	T11		T4		T8		T6	
MG PATIENTS	CORTEX	MEDULLA	CORTEX	MEDULLA	CORTEX	MEDULLA	CORTEX	MEDULLA
1	99	60	99	50	99	32	99	10
2	99	45	95	50	95	20	ND*	ND*
3	99	45	85	45	85	30	90	8
4	99	45	99	60	99	25	99	3
5	99	62	ND*	ND*	99	44	99	10
6	99	62	99	35	99	37	99	8
7	99	50	99	45	99	28	99	5
8	99	68	99	60	99	20	99	1
9	99	65	99	71	99	36	99	9
10	99	55	99	61	99	41	99	10
11	99	75	99	75	99	22	99	9
12	99	62	99	77	99	27	99	5
MEAN	99 $\pm$ 0**	58 $\pm$ 9.9	97 $\pm$ 13	57 $\pm$ 13.5	97 $\pm$ 1.3	30 $\pm$ 8.0	98 $\pm$ 0.8	7.1 $\pm$ 3.1
CONTROL								
1	99	90	99	50	99	30	99	11
2	99	70	99	75	99	30	99	10
3	99	52	99	60	99	22	99	4
	99 $\pm$ 0	71 $\pm$ 19.0	99 $\pm$ 0	62 $\pm$ 12.6	99 $\pm$ 0	27 $\pm$ 4.6	99 $\pm$ 0	8.3 $\pm$ 3.8

*NOT DONE
**Mean $\pm$ SD

TABLE II

QUANTITATION OF B CELLS IN THE THYMUS (% POSITIVE CELLS)*

	# CASES	IgD (%) CORTEX	IgD (%) MEDULLA	IgM (%) CORTEX	IgM (%) MEDULLA
MG	12	0	$9 \pm 7.0^{+}$	0	6 ± 5.4
CONTROLS	3	0	6 ± 3.5	0	7 ± 2.9

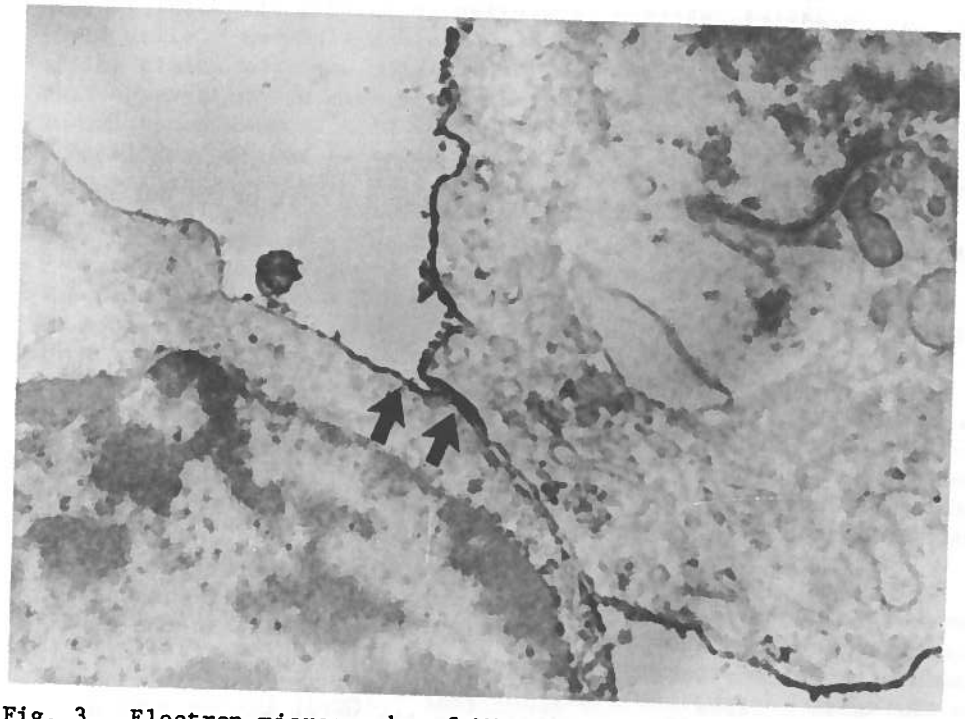

Fig. 3. Electron micrograph of MG thymus tissue sections demonstrating localization of Ia antigen. Ia positive epithelial cell (upper right) and adjacent thymic lymphocyte are shown. Lymphocyte has Ia positivity only on membrane adjacent to epithelial cell (arrows). (11,140 x)

Fig. 4. Frozen section of MG thymus demonstrating localization of keratin. Dendritic pattern is similar to pattern found for Ia antigen (see Figure 2). (100 x)

pattern was noted between MG and control thymus. The pattern of keratin localization was generally similar to that of Ia but not identical.

DISCUSSION

We have found MG and control thymus to be similar with respect to T cells and T cell subsets. The germinal centers of the MG thymus have cells with surface IgM and IgD in the same pattern as the germinal centers of lymph nodes. Scattered IgM and IgD positive cells were present in the medulla of both MG and control thymus apparently unrelated to germinal centers.

These findings in tissue sections of MG thymus differ in several respects from our previous observaions of cell suspensions from these organs.[6] The prominence of B cells in the germinal centers of MG thymus in tissue sections is not reflected in the cells suspensions where the percentage of surface immunoglobulin positive cells was similar in the MG thymus (0.5 %) and control (0.1 %). Also, the predominance of T4 positive cells over T8 and T6 positive cells in tissue sections of medulla of both MG and non-MG thymus was not reflected in the suspension data where T4 positive cells were less numerous than cells positive for either T8 or T6.[6]

The difference between data obtained from tissue sections and cell suspension may be due to several factors. Probably most important is the relative preponderance of cortical cells in the thymus. This preponderance of cortical cells in thymocyte suspensions may obscure differences between MG and control subjects in the prevalence of T4 and T8 positive cells and the germinal center B cells in the medulla of myasthenic patients. Other factors, including loss of more adherent cells in preparing thymus suspensions, heterogeneity among patients, and differences in sensitivity of techniques (immunofluorescence for cell suspension and immunoperoxidase for tissue sections) may also be involved.

In an examination of various phenotypic markers, only the Ia positive cells were significantly greater in MG patients compared to controls.[6] Since the pattern of Ia localization on frozen sections of thymus precluded meaningful identification and enumeration of positive cells by light microscopy, we examined MG thymus sections by immunoelectron microscopy to achieve better resolution. At the ultramicroscopic level, Ia antigen was localized mainly on non-lymphoid cells. Lymphoid cells had Ia positivity only on that portion of the plasma membrane adjacent to Ia positive non-lymphoid cells. This observation is similar to that reported in murine thymus[7,8] and normal human thymus.[9] In thymus cell suspensions examined by electron microscopy for Ia antigen, occasional lymphocytes had diffuse Ia positivity on their entire surface membrane and may represent either B cells or activated T cells. The vast majority of Ia positive cells examined ultrastructurally were non-lymphoid and were either epithelial cells or interdigitating dendritic cells. Using electron microscopy and immunoperoxidase techniques, we were unable to confirm the findings of a recent immunofluorescence study that found increased numbers of interdigitating dendritic cells in the MG thymus compared to control.[10] In agreement with this study, however, we have found no apparent abnormality in the distribution of T cell surface phenotypes in the MG thymus.

The non-quantitative characteristics of electron microscopic examination preclude enumeration of Ia positive cells as compared with light microscopy. Yet, the Ia positive thymic cell population appears to be heterogeneous in both MG and non-MG individuals and includes epithelial cells, interdigitating dendritic cells and some lymphocytes. Keratin appears to be a marker for thymic epithelial cells and keratin positive cells appear to have similar distributions in MG and control thymus. Additional studies using double labeling techniques are in progress and will further investigate the nature of Ia positive cells in the myasthenic thymus. Since Ia antigen is involved in T cell antigen recognition, increased numbers of Ia positive cells may be related to the autoimmune process in myasthenia gravis.

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