

Lymphocyte subpopulations in thymus and blood from patients with myasthenia gravis

JOHAN A. AARLI, PETER HEIMANN*, ROALD MATRE, SVEIN THUNOLD, AND OLAV TÖNDER

From the Departments of Neurology, Surgery, and Pathology, and the Broegelmann Research Laboratory for Microbiology, School of Medicine, University of Bergen, Norway

SUMMARY Cell suspensions were prepared from hyperplastic thymic tissue and lymphoepithelioma from patients with myasthenia gravis and from presumed normal thymic tissue obtained at cardiac surgery. The mononuclear cells were examined for surface markers. The mean percentages of both T lymphocytes and Fc receptor-carrying lymphocytes were similar in the three groups, whereas there was an increase in C receptor-carrying lymphocytes in the samples from myasthenic patients. Sections from the thymus gland were examined for T and B markers. In the hyperplastic thymus and in lymphoepithelioma, the T lymphocytes were distributed diffusely throughout the cortex and the medulla; in the normal thymus they were predominant in the cortex. The mean percentage of T and B lymphocytes in peripheral blood from patients with myasthenia gravis was normal. Thymectomy involved a transitory decrease in T lymphocytes with a corresponding increase in B lymphocytes.

The rapid variation of the muscular weakness in myasthenia gravis is commonly ascribed to the blocking effect of circulating antibodies against the acetylcholine receptor protein of the muscle endplate (Lindstrom *et al.*, 1976), while the long-term structural changes may also be caused by the effect of sensitised lymphocytes (Richman *et al.*, 1976). The neuromuscular symptoms of myasthenia gravis can, therefore, be regarded as the result of a combined humoral and cellular immune response towards antigenic determinants in the muscle endplate.

The thymus gland, which has a central role in the development of cellular immune competence, is frequently histologically abnormal in myasthenia gravis, and thymectomy can result in a significant clinical improvement. A few recent reports indicate an alteration in the distribution of T and B lymphocytes within the thymus in myasthenia gravis, but the data are conflicting (Abdou *et al.*, 1974; Lisak *et al.*, 1976; Shirai *et al.*, 1976). The aim of the present work was, therefore, to investigate whether myasthenia gravis is accompanied by a disturbance in the distribution and relative

quantities of T and B lymphocytes in the thymus, and if removal of the thymus has any effect upon T and B lymphocyte levels in the blood.

Materials and methods

PATIENTS

The patients were being treated for myasthenia gravis at the Department of Neurology. Thymectomy was performed at the Department of Surgery by the transthoracic approach. There were two patients with thymoma. One of these (MG 45) had received a seven days course of ACTH treatment (1000 iu) two months before the operation. With this exception, ACTH, steroids, or immunosuppressive drugs were not administered before thymectomy.

THYMUS

Immediately after thymectomy, parts of the organ were prepared for routine histology. Suitable pieces of the tissue were quick-frozen in liquid nitrogen for the preparation of cryostat tissue sections. The remaining tissue was minced with scissors and centrifuged at 100 g for 1 min to separate the coarser particles. The cells in suspension (thymocytes) were then washed three times in isotonic, phosphate-buffered saline, pH 7.2 (PBS). Thymic

Address for reprint requests: Professor Johan A. Aarli, Department of Neurology, 5016 Haukeland sykehus, Bergen, Norway.

*Deceased.

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tissue obtained at cardiac surgery was used as control.

PERIPHERAL BLOOD LYMPHOCYTES

Two millilitres of PBS were added to 2 ml of heparinised venous blood (10 iu/ml). The mixture was carefully layered on 3 ml of Lymphoprep (Nyegaard and Co., Oslo, Norway) and the mononuclear cells were separated by gradient centrifugation (Böyum, 1968). The cells were washed three times in PBS and kept at 4°C until use. The trypan blue exclusion test showed 95% viable cells.

DEMONSTRATION OF SURFACE MARKERS ON CELLS IN SUSPENSION

T cells were enumerated by the rosette assay with sheep erythrocytes (E) (Jondal *et al.*, 1972). Briefly, 0.1 ml of a cell suspension containing 2×10^6 cells/ml was mixed with an equal volume of a 1% suspension of sheep erythrocytes in PBS containing 20% fetal calf serum absorbed with sheep erythrocytes. After incubation at 37°C for 5 min, the cell mixture was centrifuged at 200 g for 5 min. The pellet was gently resuspended by rotating the tube along the long axis, and the percentage of rosette forming cells was counted in a haemocytometer after overnight incubation of the cell mixture at 4°C.

The indirect immunofluorescence technique was used for the demonstration of surface immunoglobulin (Ig). 0.1 ml of a cell suspension containing 4×10^6 cells was incubated with equal amounts of a fluorescein-conjugated polyvalent rabbit IgG anti-human immunoglobulin (Dakopat, Copenhagen) at room temperature for 30 min. The conjugate was diluted to a final protein concentration of 1.25 mg/ml, equal to 0.5 precipitating units/ml. The optical density ratio (OD) (OD 495/278/OD) was 0.63 ± 0.03 as specified by the manufacturer. The cells were washed three times in PBS, mounted in PBS buffered glycerol and examined for membrane fluorescence with a Leitz orthoplane microscope using epi-illumination (Beutner *et al.*, 1968). Fc receptor positive cells were demonstrated by rosette assay using ox erythrocytes (E) sensitised with rabbit IgG anti-erythrocytes antibody (A) (Hallberg *et al.*, 1973).

Erythrocytes sensitised with rabbit IgM anti-erythrocyte antibody and human complement (EAC3b) were used for the demonstration of complement receptor (C3b receptor) positive cells. The erythrocyte-antibody-complement indicator system (EAC) was prepared as described previously (Matre and Tönder, 1976). IgM-EA and E served as controls. Equal amounts of lymphocyte suspension (2×10^6 cells/ml) and indicator cells (1% suspen-

sion) were mixed and left at room temperature for one hour. After centrifugation at 200 g for 5 min and gentle resuspension of the pellet, the number of rosetting cells out of a total count of 200 was counted as for the E test. For all rosette assays, cells binding more than three indicator cells were considered rosetting cells.

DEMONSTRATION OF E, FC, AND C RECEPTORS IN TISSUE SECTIONS

The procedure used was the closed chamber technique as described by Tönder *et al.* (1974). Diffuse or focal haemadsorption was graded microscopically when the glass around the tissue was free of erythrocytes. The procedure for fixation and staining with haematoxylin and eosin was the same as described previously (Matre and Tönder, 1976).

For the demonstration of E receptor, the tissue sections were incubated at 4°C overnight using AET-treated sheep erythrocytes in PBS using 25% heat inactivated fetal calf serum. The slides were then turned over and left at 4°C for 15 min. The preparations were studied microscopically while still cold.

For the demonstration of Fc receptor, the indicator cells (EA) were prepared by sensitising sheep erythrocytes with one agglutinating unit of rabbit IgG antibodies (A). One agglutinating unit is defined as the amount of the highest dilution of antiserum which agglutinates an equal amount of a 1% suspension of erythrocytes. EA was suspended in PBS containing 0.5% normal rabbit serum. For the demonstration of C receptors, the indicator cells (EAC) were prepared as previously described (Matre and Tönder, 1976).

Tissue sections were incubated with EA or with EAC indicator cells for 30 min at room temperature.

Results

LYMPHOCYTE MARKERS ON THYMOCYTES IN SUSPENSION

Cell suspensions were prepared using hyperplastic thymic tissue from four patients with myasthenia gravis. E and EA rosette tests were performed on all four samples, EAC rosette tests only on cell suspensions from two patients (Table 1).

The mean percentage of T lymphocytes in suspension was 90 and the range 84–97% (normal 86%, range 82–90%).

The number of Fc receptor positive lymphocytes (EA rosettes) in the thymocyte suspension was in the range of 2 to 14% with a mean of 7% (normal mean 6%). Cells with C3b receptor (EAC rosettes) amounted to 25% in one sample and 9% in the

Table 1 Percentage of thymocytes with T and B lymphocyte markers in thymic tissue from four patients with myasthenia gravis and thymus hyperplasia

Patients	T lymphocyte markers (%) E rosettes	B lymphocyte markers (%)		
		EA rosettes (1 aggl. unit)	C3b receptor	Surface Ig
MG 22	97	2	ND	ND
MG 38	88	6	ND	ND
MG 41	90	7	25	ND
MG 46	84	14	9	<1
Normal thymus (mean values)	86	6	6	<1
Normal blood	75	28	25	14

ND=not done.

other, which were higher than in normal thymus (mean 6%).

A small thymoma of the lymphoepithelial type (15×5×5 mm) was present in the thymus removed from patient MG 45. The tumour was easily dissected free and a tumour cell suspension prepared. Ninety per cent of the cells formed E rosettes, indicating that the tumour consisted mainly of T lymphocytes. Patient MG 44 had an infiltrating lymphoepithelioma, and 92% of the thymocytes from this tumour formed E rosettes. The small population of B lymphocytes present in these two tumours seemed to consist mainly of cells with complement receptor (Table 2). On the other hand, the T and B lymphocyte percentages, both in the hyperplastic thymus and the lymphoepithelioma, did not vary from data obtained with apparently normal thymus tissue.

CELL MARKERS IN TISSUE SECTIONS

When the tissue section technique was performed using normal thymus tissue, the E indicator cells adhered mainly to the cortex, less to the medulla and not at all to the Hassall's corpuscles. In the thymoma, the E indicator cells adhered diffusely to the tissue section (Fig. 1a), while the EA and EAC3b indicator cells were scattered throughout the tissue (Fig. 1b). However, the EAC3b adhered

Table 2 Percentage of thymocytes with T and B lymphocyte markers in thymoma (lymphoepithelioma) from two patients with myasthenia gravis

Patients	T lymphocyte markers (%) E rosettes	B lymphocyte markers (%)		
		EA rosettes (1 aggl. unit)	C3b receptor	Surface Ig
MG 44	92	7	14	<1
MG 45	90	4	14	2-3

mainly to some small areas in the tumour (Fig. 1b) which did not bind E (Fig. 1a) or EA indicator cells. The nature of the cells within these areas could not be identified with certainty in frozen sections stained with H and E. Definite germinal centres were not detected in frozen sections of the thymoma.

PERIPHERAL T AND B LYMPHOCYTES

Pre-thymectomy values

E, EA, and EAC rosette tests were performed using suspensions of peripheral blood lymphocytes from seven myasthenia gravis patients before thymectomy. The number of E rosetting lymphocytes was within the normal range (58 to 73%) in all patients, and the mean was 68%. The mean percentage of EA rosette-forming lymphocytes was 22% (range 12 to 31%). EAC rosette-forming lymphocytes varied from 13 to 50%, (mean 27%). Except for a high number of EAC rosette-forming lymphocytes in one patient, who also had an adenocarcinoma of the colon, the numbers of circulating T and B lymphocytes were within the normal range in all patients.

Post-thymectomy values

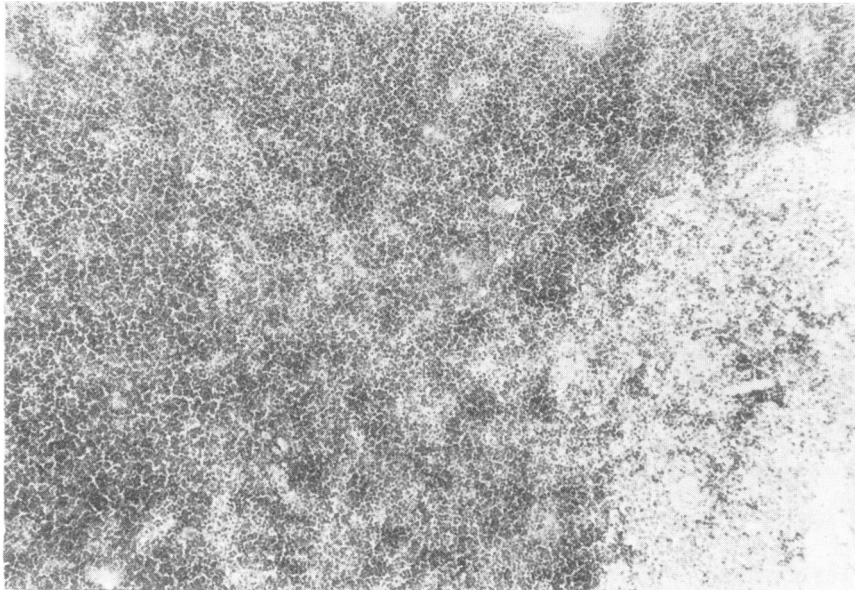
Peripheral blood was drawn from the patients two and six days after the thymectomy, and then at irregular intervals during the first two to 12 weeks after operation. E and EA rosette tests were performed on all samples, EAC rosette tests on five (MG 41, 42, 44, 45, and 46).

In all samples, the percentage of E rosetting lymphocytes was slightly decreased, and the number of EA rosetting lymphocytes increased. The fall in the first population corresponded grossly to the increase in the second population (Fig. 2). Also, EAC rosette-forming lymphocytes and Ig-positive lymphocytes increased in numbers which corresponded to the moderate increase in EA rosetting lymphocytes. The changes in the relationship between the cell populations were transitory, since the percentage of E, EA, and EAC-forming lymphocytes two to three months later were approximately identical with preoperative values.

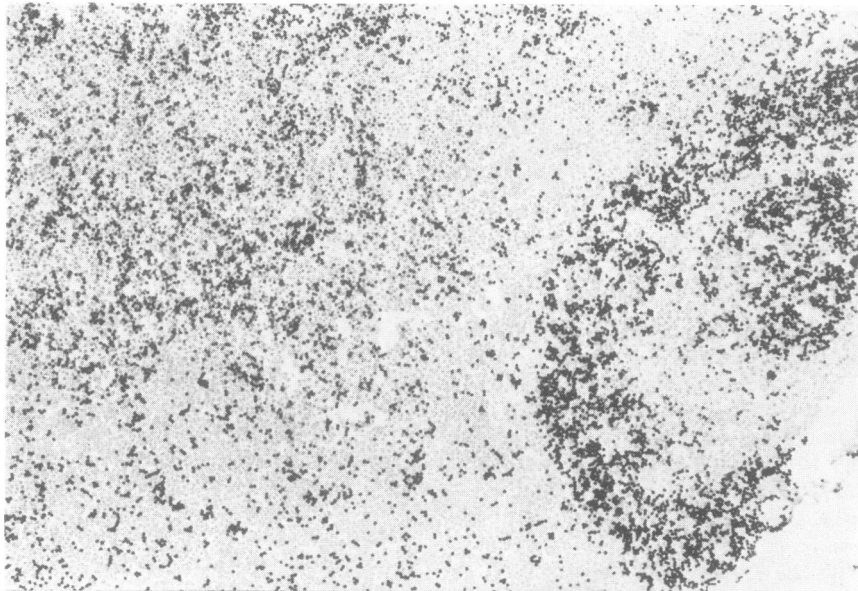
Blood was also obtained from three female patients who underwent thymectomy three, seven, and nine years previously, and who had full remission of clinical symptoms. The percentage and absolute numbers of T lymphocytes, EA, EAC3b-rosetting, and surface Ig-lymphocytes were normal.

Discussion

Approximately 90% of the cells from sus-



(a)



(b)

Fig. 1 *Haemadsorption to tissue sections of a thymoma adsorption of E and EAC3b indicator cells to cryostat sections (H and E, $\times 112$). (a) The E cells adhere in a uniform pattern. (b) EAC3b are scattered throughout the tissue. Note area far right which is negative with E cells and positive with EAC3b cells.*

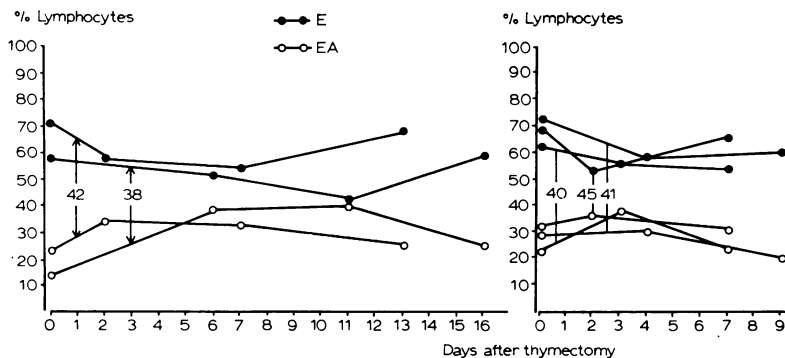


Fig. 2 Results of E—●—●— and EA—○—○— rosette tests with lymphocytes from peripheral blood of five patients with myasthenia gravis before and after thymectomy. Left: MG 42 and MG 38, right: MG 40, 41, and 45.

pensions of hyperplastic thymus of myasthenia gravis patients were T lymphocytes. This figure is not significantly different from that obtained using cell suspensions of normal thymic tissue. Even in cell suspensions from two thymomas of lymphoepithelial type, the percentage of T lymphocytes was normal. Similar data have also been reported by Abdou *et al.* (1974) and Shirai *et al.* (1976), and it can now, presumably, be concluded that the percentage of T lymphocytes both in thymoma and hyperplastic thymic tissue in myasthenia gravis is normal.

There was, however, a difference in the distribution of T lymphocytes within the thymus. In the hyperplastic thymus and in the lymphoepithelioma, the T lymphocytes were distributed diffusely throughout the cortex and medulla, whereas in the normal thymus they were predominant in the cortex.

The thymus gland has also a small subpopulation of B lymphocytes (Birnbau and Tsairis, 1977). Our data indicate that the number of C receptor-carrying thymocytes is higher in the myasthenic thymus than in the normal thymus. Twenty-five per cent and 9% of the thymocytes from the two hyperplastic thymuses and 14% of the thymocytes from each of the two lymphoepitheliomas formed rosettes with EAC indicator cells. These percentages are significantly higher than those obtained using cell suspensions of normal thymic tissue. In the normal, and also in the hyperplastic thymus, the C receptor-carrying cells were not strictly confined to follicles in the medulla, as reported by Staber *et al.* (1975), but scattered diffusely throughout the tissue. In thymoma sections, however, these cells were clustered in small areas almost devoid of T lymphocytes and Fc receptor-carrying lymphocytes. These areas were apparently not germinal centres, but their histological nature could not be defined.

In contrast, the percentage of Fc receptor-carrying lymphocytes, both from the thymoma and

the hyperplastic thymic tissue, did not differ significantly from that of normal thymic tissue. The increase in the B lymphocyte subpopulation in the thymic tissue from myasthenia gravis patients consisted mainly of C receptor-carrying lymphocytes, which in the thymoma occurred in clusters. The relative and absolute numbers of C and Fc receptor-carrying lymphocytes in peripheral blood were similar in myasthenia gravis patients and healthy control subjects. The lower level of Fc receptor-carrying lymphocytes reported by Lisak *et al.* (1976) is presumably accounted for by the use of different techniques by the two groups.

The mean percentage of T lymphocytes in peripheral blood from myasthenia gravis patients was 68%, which is normal. These data agree with those reported by Abdou *et al.* (1974) and Kallden *et al.* (1976).

Thymectomy resulted in a slight decrease in circulating T lymphocytes with a corresponding increase in B lymphocytes. This reaction was transitory and the values returned gradually to pre-operative levels. Similar findings have also been reported by Fritze and Herrmann (1975), Sandilands *et al.* (1975), and Pudifin and Cox (1976). Björkholm *et al.* (1975) found that a significant decrease in the number of T lymphocytes might appear several years after thymectomy. However, Bowen and Pabst (1976) found no indication of immuno-deficiency in myasthenia gravis patients who had undergone thymectomy. The long-term effect of thymectomy on the immune competence in patients with myasthenia gravis is still unknown.

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