

Comparative analysis of T and B-cell subpopulations in normal
thymus and thymus of myasthenia gravis

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INTRODUCTION

Considerable progress has been made recently in the study of cell differentiation mediated by the thymus gland, particularly through studies conducted on mice (Miller¹⁾, Hirokawa²⁾). The objective of this study has been to extend our understanding in this field by identifying some characteristics of functional disorders in the human thymus, namely:

A) inhibition of the maturation process in T-cells, and/or the possibility of a disorder of 'barrier mechanism' working against B-cell infiltration into the thymus of myasthenia gravis; and, B) sensitivity to ATS for myasthenic thymocytes compared with the conditions of normal, healthy thymus cells. The scope of this report has been limited to the pathological characteristics of human thymus, studied through patients with one type of thymus disorder, i.e. those suffering myasthenia gravis.

Although there are several studies^{3),4)} related to the cell constitution of normal thymus, there are no previous studies investigating disorders of thymus of myasthenia gravis. Here the subpopulation of T and B-cells of myasthenia gravis and normal thymus are examined concurrently and on a comparative basis.

MATERIALS AND METHODS

1) Preparation of thymocytes

Thymocytes were obtained from 10 myasthenic patients who had not been treated with corticosteroid therapy, but who had received thymectomy for treatment. Control thymocytes were obtained from congenital heart disease patients during open heart surgery. These thymus cells were cut, ground on a stainless steel mesh, and then suspended. Nucleated cells were purified in the suspension by the Ficoll-Hypaque method.⁵⁾ The cell number of samples was set for these tests at 5×10^6 /ml.

2) Preparation of anti-thymocyte serum (ATS)

Normal, healthy thymus cells were obtained as described above. Volumes of 1 mM EDTA were added and mixed for 2 mins. at 37°C. To this solution a large volume of phosphate buffer

saline was added to stop any more reaction. This fluid was centrifugified, and the supernatant was then used as the membrane antigen of thymocytes. The quantity of this antigen was estimated by Coleman's spectrometer (Hitachi, Tokyo, Japan). One mg of this antigen, emulsified in Freund's complete adjuvant was injected into a rabbit intracutaneously, two times, at one week's interval. One week following the last injection, 0.5 mg of an alum precipitated form of this antigen, was then injected intravenously into the same animal. One week later the blood was harvested as the antithymocyte serum. This serum was inactivated at 56°C for 30 mins. and absorbed thoroughly with human red blood cells (AB group), as well as the cultured cells of B-cell human leukemia patients. This serum was then used for the cytotoxicity test with guinea pig serum as the complement.

a) T lymphocytes

i) E-rosette formation method.

0.2 ml of thymus cell samples were mixed in equal volume with sheep red blood cells (SRBC at 1×10^8 /ml concentration) and centrifugified at 1000 rpm for 10 mins at room temperature. After being brought to 4°C and left for an hour, this mixture was re-suspended and the E-rosette forming cells were counted microscopically. Lymphocytes that combined with more than four SRBC were counted as T-E (+) cells.

ii) Cytotoxicity test with anti-thymocyte serum.

Anti-thymocyte serum (ATS), obtained as described previously, was diluted to two different consistencies, i.e. 1:16 and 1:64. Then 50 μ l of each dilutant, including 40 μ l of ATS and 10 μ l of complement, were mixed together with the cell samples. The mixture was left for 45 mins at 37°C. By this procedure the living cell count could be made microscopically using the dye exclusion method.^{6),7)}

b) B lymphocytes: EAC-rosette formation method.⁵⁾

19S antibody was separated from the anti-SRBC serum prepared previously using Sephadex G-200 and then diluted. This was then mixed with an equal volume of SRBC in suspension (2×10^9 /ml) and left for 30 mins at 37°C. This stage was followed by washing in Galetin-Veronal buffer (GVB) and re-adjusted again to 2×10^9 /ml of SRBC. A tenth volume of fresh C57 BL/10 mouse serum was added and then left for 15 mins at 37°C. After washing with GVB, it was again re-adjusted to a concentration of 5×10^8 /ml of SRBC. 0.2 ml of this mixture (EAC) was added to an equal volume of thymocyte cells in suspension, and left for an hour at 37°C. After being brought to 4°C and left for an hour, the EAC cells were counted microscopically. Lymphocytes that combined with more than four EACs were counted as B-EAC (+) cells.

RESULTS

1) Specificity of ATS

Cytotoxicity of ATS was shown to be effective on control thymocytes even if diluted 500 or 1000 fold (shown in Fig. 1). 50–60% normal peripheral lymphocytes were killed by this serum. Cultured T-leukemia cells were also killed (100%) by this serum. However B-leukimia cells were found to be resistant to this serum.

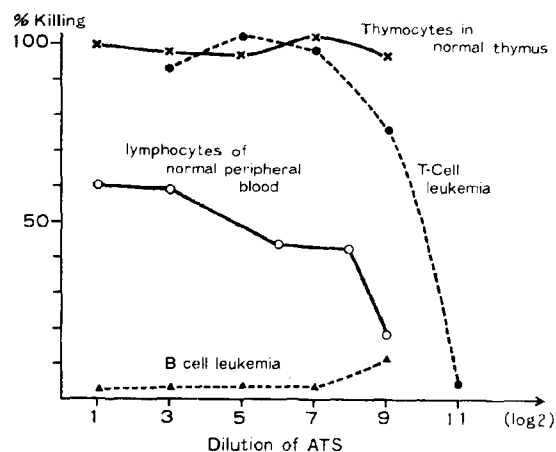


Fig. 1 ATS showed sufficient cytotoxic effects upon normal thymocytes ($\times-\times$), and T cell leukemia ($\circ-\circ$). On the other hand it didn't show any cytotoxic effect upon B cell leukemia ($\triangle-\triangle$).

Table. 1 T and B Cell subpopulations for Normal Thymus

Case No.	Disease	T-Cells		B-Cells
		T-ATS (%)	T-E (%)	B-AEC (%)
1. 5 year-old male	ASD	96	84	0
2. 8 year-old male	ASD	n.d.*	77	1.3
3. 32 year-old male	ASD	99	70	2.6
4. 40 year-old male	MS	98	71	0
5. 8 year-old female	VSD	96	70	3

* not done

2) T and B-cell subpopulation in normal thymus

The percentages of T and B-cells found in normal, healthy thymus are shown in Table 1. In four cases (Nos. 1, 3, 4 and 5) thymocytes were killed, thus indicating that 96–99% of normal human thymus consist of ATS sensitive cells (T-ATS). However, with the E-rosette formation method (T-E), only 70–84% were detected in all cases. The number of B-cells detected as EAC rosettes was 0–3%.

3) T and B-cell subpopulations in myasthenic thymus

Ten cases of T and B-cell subpopulations were investigated from myasthenia gravis patients (Table 2). In 3 of these cases (Nos. 1, 2 and 10), due to the small thymoma in their thymuses, cells were obtained and counted from both their thymomatous as well as non-thymomatous areas of the thymus. In 2 cases (Nos. 5 and 6), since almost the whole of their thymuses were occupied by large thymoma, cell samples were obtained only from those thymomatous areas.

It can be seen from Table 2 that the proportion of B-cells (B-EAC) was significantly higher at 10–30% for all myasthenic thymus, as compared with that of normal thymus. Conversely, the T-cell proportion was somewhat lower than that of normal thymus cells.

In normal (control) thymus, the T-ATS value was high (more than 96%), as shown in Table 1. The T-E value was also high, although usually about 20–30% lower than that of T-ATS. On

Table. 2 T and B Cell subpopulations in myasthenia thymus

Case No.	Clinical Classification of M.G (Ossaman's)	Complication of thymoma	T-Cell		B-Cell	
			T-ATS (%)	T-E (%)	B-EAC (%)	
1. 20 year-old male	III	(+)	* [1)	86	76	n.d.
			* [2)	88	75	15
2. 22 year-old male	IIA	(+)	* [1)	70	n.d.	n.d.
			* [2)	83	n.d.	n.d.
3. 24 year-old female	IIB	(-)		92	69	1.3
4. 53 year-old male	IIB	(-)		72	n.d.	n.d.
5. 28 year-old female	IIB	(+)	**	78	76	10
6. 65 year-old male	IIB	(+)	**	n.d.	40	35
7. 28 year-old female	IIB	(-)		n.d.	65	30
8. 20 year-old female	IIB	(-)		n.d.	75	12
9. 31 year-old female	IIB	(-)		81	38	13
10. 53 year-old male	IIA	(+)	* [1)	69	42	22
			* [2)	43	65	12

* Thymus complicated with thymoma was evaluated about their thymocytes obtained from thymomatous area 1), and from non-thymomatous area 2).

** Cells were obtained only from thymomatous area because thymoma was large enough to occupy almost part of thymus.

n.d.: not done.

the other hand, it seems that a dissociation exists between the T-ATS and T-E values of myasthenic thymus. No. 9, for example, shows a T-E value of 43% while there were 81% T-cells detected by ATS. For No. 10, on the other hand, the T-ATS value was smaller than that of the T-E value. Cells, however, from thymomatous and non-thymomatous thymus did not differ.

4) Dilution of ATS and it's cytotoxic effects on both normal and myasthenic cells

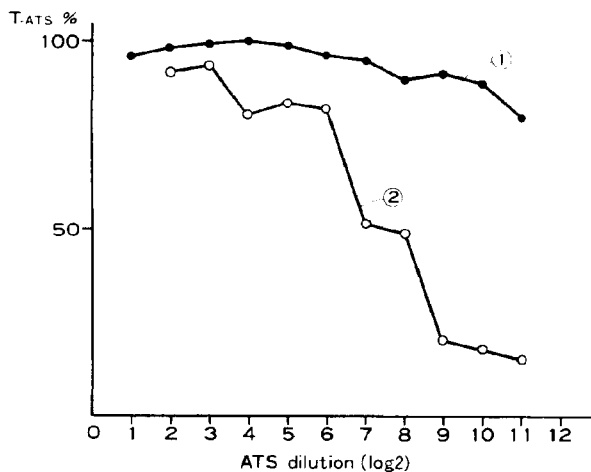


Fig. 2 In most cases, as shown by 1. The T-ATS value in normal thymus was still high until the ATS was diluted to low concentration. But in only one case, as shown by 2, the ATS value rapidly decreased even when the ATS was diluted not so much.

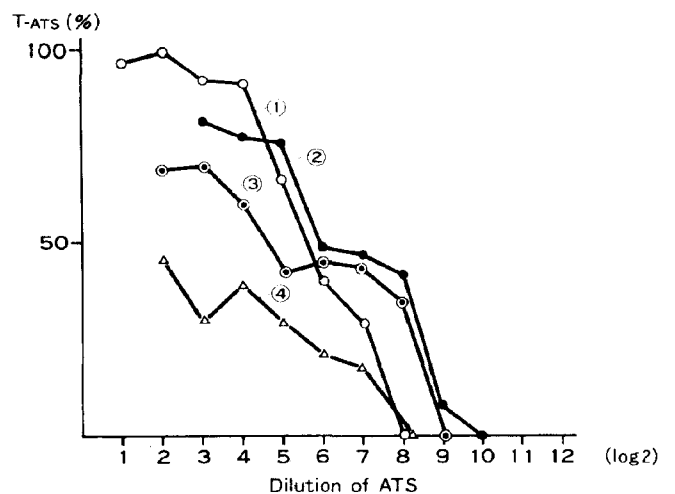


Fig. 3 The T-ATS percentage of myasthenia showed a comparatively lower cell count at high concentration of ATS. The cells in all cases increase their resistance at low concentration.

Fig. 2 shows the percentages of T-ATS for normal (control) thymus cells when ATS was diluted. As shown by graph path No. 1 the T-ATS proportion remained more or less constant until diluted 1:512. In one, exceptional case, the cells were resistant to the killing by low concentrations of ATS, as indicated by graph path No. 2, Fig. 2. On the other hand, as shown in Fig. 3 T-ATS percentage of myasthenic cells, in 3 out of 4 cases, showed a comparatively lower cell count at high concentrations of ATS. The cells in all cases increase their resistance to ATS at low concentrations.

DISCUSSION

It has been demonstrated, by others, that the pathological incidence of thymoma is high in myasthenia. Up to the present time immunological studies of myasthenic thymus have not achieved much. This investigation has been conducted so as to make a comparative analysis of the T and B-cell subpopulations in both normal and myasthenic thymuses with the purpose of identifying some aspects that are characteristic of thymus disorders.

These results indicate the following:

1) In normal thymus, the proportion of T-cells are very high, while the B-cell population is almost negligible. The mean proportion of T-cells shown to be T-ATS was 97%, while those found to be T-E were 74%. Thus T-ATS was 12–24% higher than that of T-E, including all normal cases.

On the other hand, the proportion of B-cells (B-EAC) in the myasthenic thymus was significantly higher in contrast with normal (control) thymuses. Also a large number of Ig bearing cells were detected in the analysis of the myasthenic cells by the immunofluorescence method (unpublished data). Parkman,³⁾ whose work on the analysis of human embryo thymocytes showed that the thymocytes of the embryo up to the eight week demonstrated EAC-rosette (+) and E-rosette (—) thymocytes. According to their results, the existence of the large number of B-cells in myasthenic thymus may be due to some disorders in the maturation of thymus. It seems possible that there may be some 'barrier mechanism' which prevents the invasion of B-lymphocytes into the thymus tissue in normal, healthy thymus cells, while for myasthenic thymus some other disorder in the mechanism may promote, or rather, reduce the inhibitory process that controls the B-lymphocytes' entering the thymus tissue.

2) A further aspect resulting from this investigation was the apparent and significant dissociation of the T-ATS and T-E percentages in the myasthenia samples. In normal thymus the cells were sensitive to low concentrations of ATS (Tables 1 and 2). Whereas in myasthenic cases cells were resistant to the low concentration of ATS. However, one case of myasthenia showed almost as high a T-ATS proportion as normal thymus at high concentrations, although cells of this case were resistant when diluted by only 4 or 8 times. It may be that for myasthenia gravis, the surface determinants of the thymocyte antigen may be different from the surface receptor for SRBC, and that the thymocyte antigen may also be different from that of normal thymocytes in their quantity and/or quality.

SUMMARY

A clinico-pathological investigation was performed, examining T and B-cell subpopulations of normal and myasthenia thymus.

The proportion of T-cells in healthy human thymus was found to be high (96–99%), as detected by the cytotoxicity method using the antithymocyte serum plus complement (T-ATS). A second test, likewise, confirmed this with similar results, using the E-rosette method with SRBC (T-E). B-cells in healthy thymus were low (0–3%), when measured by the EAC-rosette method employing SRBC and anti-SRBC antibody with mouse complement.

However, in tests on myasthenic thymus cells the proportion of T-cells was low (43–88%). On the other hand, B-cells were found to be significantly higher (10–35%) than for healthy thymus cells. Furthermore, the use of these two testing methods, i.e. T-E and T-ATS, provided a noticeable difference between their results in the total T-cell counts for each group.

A further item of interest was detected by the dissociation between the proportion of T-ATS and T-E for myasthenic cells. In most cases, healthy thymus cells were sensitive to even the lowest concentrations of ATS (1:2,408). On the other hand, some myasthenic thymus cells seem to have a low amount of thymic antigen, even at high concentrations, since they were not killed by higher dilutions of ATS. This tendency was similar for both cell samples collected from thymomatous and non-thymomatous areas of myasthenic patients.

REFERENCES

- 1) Miller, J. F. A. P.: Immunological function of the thymus, *Lancet* 2: 748–749, 1961.
- 2) Hirokawa, K.: The thymus and aging. *In* *Comprehensive Immunology*. Vol. I. ed. Good, R. A. and Day, S. B. Plenum Book, New York: 51, 1977.
- 3) Parkman, R.: Treatment of immunodeficiency diseases by organ transplantation. *In* *Progress in clinical immunology*. Vol. 3. ed. Schwartz, R. S. Gune and Stratton, New York: 85, 1977.
- 4) Good, G. A. and Bach, F. H.: Bone marrow and thymus transplantation. Cellular engineering to correct primary immunodeficiency. *In* *Clinical immunobiology*. Vol. 2. ed. Good, R. A. and Bach, F. H. Academic Press, New York: 63, 1974.
- 5) Winchester, R. and Ross, G.: Method of enumerating lymphocyte subpopulation. *In* *Manual of clinical immunology*. Vol. 1. ed. Rose, B. R. and Fiedman, H. American society for microbiology. Washington: 64, 1976.
- 6) Yodoi, J. et al.: Anti-human T cell serum. *Rinshokagaku*, 11: 45–60, 1975.
- 7) Sarker, S. et al.: A rabbit antiserum to a thymus extract specific for mouse thymus. *J. Immunol.*, 110: 1222–1229, 1973.