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T and B lymphocytes in myasthenia gravis

Y. ITOYAMA, S. KAWANAMI, I. GOTO & Y. KUROIWA *Department of Neurology, Neurological Institute, Kyushu University, Division of Neurology, Fukuoka University Hospital, Fukuoka, Japan*

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SUMMARY

Peripheral blood lymphocytes from seventeen non-thymectomized and nine thymectomized patients with myasthenia gravis (MG) and thirteen healthy controls were examined for the presence of surface markers characteristic of T and B lymphocytes by rosette formation with sheep red blood cells (SRBC). T cells were identified by their capacity to spontaneously form rosettes with SRBCs. The percentage of B lymphocytes was determined by the erythrocyte antibody complement (EAC) rosette-forming test. The EAC complex was prepared with either whole rabbit anti-SRBC serum or with the IgM fraction of rabbit anti-SRBC serum. The two kind of erythrocyte complement rosette-forming cells (EAC-RFC) are designated erythrocyte-haemolysin-complement RFC (EA(H)C-RFC), and erythrocyte-IgM-complement RFC (EA(M) C-RFC). The percentage of total lymphocytes and T cells was not altered in MG patients. The percentage of 'active' T cells, which have been considered to be more actively involved in cellular immunity, was also similar in MG patients and controls. A significant increase in EA(H)C-RFC occurred in both thymectomized and non-thymectomized MG patients, while in B cells detected by EA(M)C-RFC no alterations were found. The increase in EA(H)C-RFC in lymphocytes from MG patients may be due to an increase in the 19S antibody-forming B lymphocytes or to an increase in T cells which have Fc receptors on their surface.

INTRODUCTION

The cause or causes of myasthenia gravis are not known. Evidence exists that supports the involvement of the immune system in the aetiology of the disease. High titres of circulating antibodies directed against muscle (Straus *et al.*, 1960), thymus and acetylcholine receptors (Bender *et al.*, 1975; Monnier & Fulpius, 1977) have been found in MG patients. A decrease in complement accompanies exacerbations of the disease state (Nastuk, Plestia & Osserman, 1960). Lymphorrhages in muscle (Russell, 1953) and thymic pathology (Sloan, 1943) are common in MG patients. These findings, along with the beneficial effects of thymectomy in MG patients (Papatestas *et al.*, 1971), suggest that autoimmunity plays a role in the pathogenesis of this disease.

T lymphocytes are readily identified on the basis of their ability to form spontaneous rosettes with SRBC (Yata, Tsukimoto & Tachibana, 1973). Recently, rosette-forming cells which react with SRBC during short periods of incubation have been described to be more actively involved in cellular immunity and have been called 'active' T cells (Wybran & Fudenberg, 1973; Jondal *et al.*, 1973). B lymphocytes can be identified by the presence of complements, IgG Fc receptors or immunoglobulins on their surface (Jondal, Holm & Wigzell, 1972).

The purpose of this study was to elucidate whether the percentage of circulating B cells, T cells or subpopulations of T cells are altered in MG patients. In addition, the effects of thymectomy on these lymphocyte subpopulations were also investigated in MG patients.

Correspondence: Dr S. Kawanami, Division of Neurology, Fukuoka University Hospital, 34 Nanakuma, Nishi-ku, Fukuoak, Japan 814

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MATERIALS AND METHODS

Patients. Twenty-six patients with myasthenia gravis (MG) ranging in age from 17 to 45 years were divided into two groups: thymectomized (nine patients) and non-thymectomized (seventeen patients). The length of time since thymectomy ranged from 2 weeks to 56 months, with a mean of 19 months. Thirteen healthy controls were also studied. None of the subjects had received corticosteroids, immunosuppressants or ACTH.

Lymphocyte isolation and characterization. Ten ml of peripheral blood were collected in a heparinized syringe. Lymphocytes were isolated by Conray–Ficoll gradient centrifugation and monocytes were excluded during the centrifugation by prior incubation with carbonyl iron powder. The isolated cells were washed and total and differential white blood cells count were done.

The percentage of T lymphocytes was determined by the erythrocyte rosette-forming test. A 0·25 ml suspension of lymphocytes (1×10^6 cells/ml) was mixed with an equal volume of sheep red blood cells (SRBC) (2×10^8 cells/ml) which were suspended in heat-inactivated foetal calf serum previously absorbed by SRBC. Two incubation times were used. Firstly, immediately following the addition of SRBC the lymphocytes were pelleted at 90 *g* for 5 min. The percentage of lymphocytes forming rosettes with four or more SRBCs represented the active T cell population. Secondly, a 15 min incubation at 37°C preceded the centrifugation and the cells were then stored on ice for 4 hr or overnight prior to counting. After the supernatant was removed, the pellet was gently resuspended by shaking. One drop of the cell suspension was mounted on a glass slide, covered by a cover slip and sealed. The percentage of lymphocytes forming rosettes with at least four SRBCs comprised the total T cell population. All samples were performed in duplicate and a minimum of 200 lymphocytes were counted for each test.

The percentage of B lymphocytes was determined by the erythrocyte antibody complement (EAC) rosette-forming test. The EAC complex was prepared with either whole rabbit anti-SRBC serum (Kyokuto Co., Tokyo) or with the IgM fraction of rabbit anti-SRBC serum (Cordis Laboratories, Miami, Florida). SRBC (1×10^8) were incubated with an equal volume of the antiserum (whole anti-SRBC serum or the IgM fraction) at a subagglutinating dilution for 30 min at 37°C. Mouse serum from the CF-1 strain was added as a source of complement. This serum was diluted 1 : 10 and the incubation was at 37°C for 30 min. After three washes, a 1×10^8 cells/ml suspension of SRBC coated with the complement and either whole anti-SRBC serum or the IgM fraction of anti-SRBC serum were prepared and designated EA(H)C or EA(M)C, respectively. The percentage of B lymphocytes was determined in each patient using EAC complexes prepared with both antisera. Following a 15 min incubation at 37°C, the cells were centrifuged at 90 *g* for 5 min, incubated again at 37°C for 30 min and then resuspended by vigorous mixing. The percentage of lymphocytes binding four or more SRBC was determined by counting a minimum of 200 cells. All tests were performed in duplicate.

RESULTS

E-rosette-forming cells (E-RFC) (Table 1)

The mean percentage of total T cells was 73·7±8·2 in patients with MG and 76·4±4·4 in controls. The difference was not significant. In sixteen patients with MG, the mean percentage of active T cells was 23·6±5·0 compared with 25·2±6·3 in controls, and these values were not significantly different. There was no difference between thymectomized and non-thymectomized MG patients, comparing the percentages of both active and total cells. There was a tendency for the values of active and total T cells to be smaller in the ocular type than in those of the generalized type, but the difference was not significant.

TABLE 1. T lymphocytes in myasthenia gravis

	n	Active T (%)	Total T (%)
Control	15	25·2±6·3*	76·4±4·4
MG (total)	16	23·6±5·0	73·7±8·2
Thymectomized	5	26·0±7·0	77·9±4·7
Non-thymectomized	11	22·5±3·6	73·1±8·0
Ocular type	4	22·5±4·3	72·6±6·3
Generalized type	12	24·9±6·0	74·4±9·3

* Mean±s.d.

MG = Myasthenia gravis.

n = Numbers of patients or normal controls examined.

EAC-rosette-forming cells (EAC-RFC) (Table 2, Fig. 1)

The percentage of EA(H)C-RFC in the group of non-thymectomized and thymectomized MG patients were 28·1±8·2 and 30·2±5·7, respectively. These values were significantly increased ($P<0·05$) in comparison with those of controls, 22·3±4·6%. (Fig. 1). In eight patients with thymoma, the EA(H)C-RFC was 31·9±7·4%. These values were all significantly increased in comparison with those of the controls. The values were slightly increased in MG patients with thymoma compared with those without thymoma.

The percentage of EA(M)C-RFC in non-thymectomized and thymectomized MG patients were 18·3±5·6 and 15·2±4·6, respectively. The value for normal controls was 16·0±6·2%. There was no significant difference among these three groups, nor between non-thymectomized and thymectomized MG patients.

TABLE 2. Lymphocyte subpopulations in myasthenia gravis

	n		B cell EA (H) C-RFC	B cell EA (M) C-RFC	T cell E-RFC	Total lymphocytes
Control	13	%	22.3±4·6*	16·0±6·2	76·6±4·0	
		a.n.	486±166	354±167	1692±524	2207±676
MG						
Non-TX	17	%	28·1± 8·2†	18·3±5·6	72·9±10	
		a.n.	559±208	367±159	1692±524	2000±543
TX	9	%	30·2± 5·7†	15·2±4·6	75·5±4·5	
		a.n.	694±215	374±168	1767±518	2343±685
Tm	8	%	31·4± 6·5†	13·6±2·3	72·0±7·7	
	7	a.n.	708±97	394±49	1561±201	2118±251

* Mean±s.d.

† $P<0·05$ by Student's *t*-test.

MG = Myasthenia gravis, non-TX = non-thymectomized, TX = thymectomized, Tm = thymoma.

n = Number of patients or controls.

a.n. = Absolute number of cells/mm³.

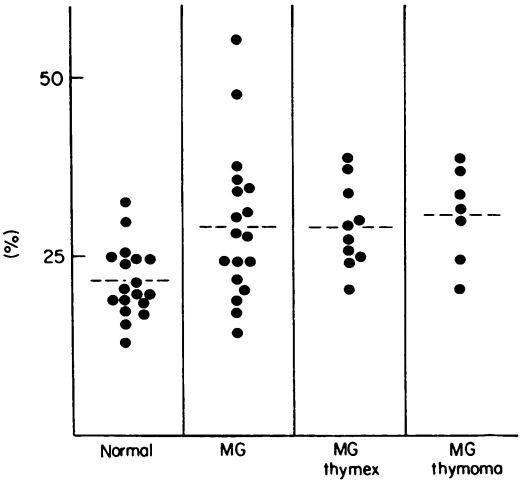

FIG. 1. Percentage of B cells detected by EA(H)C rosette-forming cells. MG = Myasthenia gravis, thymex = thymectomized. The dotted line shows the level of mean value.

Absolute number of lymphocytes (Table 2)

The total number of peripheral lymphocytes in seventeen non-thymectomized MG patients was $2000 \pm 543/mm^3$, which was not significantly lower than that of controls ($2207 \pm 676/mm^3$) or thymectomized patients ($2343 \pm 686/mm^3$).

DISCUSSION

Previous investigations of the peripheral blood lymphocytes in MG patients have not revealed any consistent findings regarding alterations in lymphocyte subpopulations. Adbou *et al.* (1974) found no significant differences between four MG patients and controls. Kozier, Bloch & Perlo (1971), investigating ten non-thymectomized patients with MG, also found no alterations in lymphocyte subpopulations, although they did find a significant decrease in the percentage of T cells forming rosettes with three or more SRBC in fifteen thymectomized patients, and total numbers of T and B cells had also decreased. These patients had received radiation therapy prior to or following thymectomy and this may have contributed to alterations in the peripheral blood lymphocyte subpopulations. In two Japanese studies (Nakata, Tada & Arimori, 1974; Kazi, 1975), the mean percentage of T cells was increased in thymectomized MG patients and in patients with generalized myasthenia. Nakata *et al.* (1974) also found the mean percentage of surface immunoglobulin bearing B cells to be increased in thirteen thymectomized patients. Our results demonstrated an increase in B cells detected by EA(H)C-RFC. This increase was found in both thymectomized and non-thymectomized patients. However, no alteration in the percentage of EA(M)C-RFC was observed in the peripheral blood of MG patients.

Human B lymphocytes can be identified by their receptors for the Fc portion of IgG or for complement. When the EAC complex was prepared with the IgM fraction of anti-SRBC serum, the SRBCs bind only to the complement receptor on lymphocytes (Holm *et al.*, 1975). If the EAC complex is prepared with whole antiserum or the IgG fraction, the SRBCs appear to bind the receptor for the Fc portion of IgG and for the complement receptor on lymphocytes. Substantial portions of both 19S and 7S antibody-forming B lymphocytes have Fc receptors. Only 7S antibody-forming cells, however, carry complement receptors (Parish & Hayward, 1974). Gergely *et al.* (1977) investigated the frequency of Fc-positive B lymphocytes in human blood. The proportion of Fc receptor-positive cells within the surface immunoglobulin carrying population was estimated to be between 11·8 and 36·2%. Therefore, EA(M)C-RFC were detecting only 7S antibody-forming B cells and EA(H)C-RFC were composed of both 19S and 7S antibody-forming cells. Our results support this finding as control patients had a higher percentage of EA(H)C-RFC ($22{\cdot}3 \pm 4{\cdot}6\%$) than EA(M)C-RFC ($16{\cdot}0 \pm 6{\cdot}2$). Similar results have been obtained by Holm *et al.* (1975). Since the increase in B cells was only detected by EA(H)C-RFC, our results suggest that an increase in 19S antibody-forming cells may occur in MG.

Recently, T lymphocytes detected after short periods of incubation have been described to be more actively involved in cellular immunity than the total T cell population and these cells have been called 'active' T cells (Wybran & Fudenberg, 1937; Jondal, Wigzell & Aiuti, 1973). In our studies neither active nor total T cells in MG patients differed from controls. When T cells were activated with isoantigens they demonstrated Fc receptors on their surface (Yoshida & Anderson, 1972). In MG patients, thymocytes and peripheral blood lymphocytes have been reported to be sensitized by muscle antigen (Alpert *et al.*, 1972), acetylcholine receptors (Abramsky *et al.*, 1975) and thymus tissue antigens (Goust, Castaigne & Montias, 1974; Kawanami, Itoyama & Kuroiwa, 1976). The increase in the B lymphocytes of MG patients detected by EA(H)C-RFC may be due to an increase in T lymphocytes which have been activated and contain Fc receptors. These cells have been referred to as 'helper' T cells (Parish, 1975).

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