

Subpopulations of T lymphocytes in myasthenia gravis patients

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SUMMARY

Subpopulations of human peripheral blood T lymphocytes were examined in twenty-three myasthenic patients. T lymphocytes bearing receptors for the Fc portion of IgG (T_γ) were significantly increased in a third of the patients examined. T lymphocytes bearing receptors for the Fc portion of IgM (T_μ) were within normal values in all but two patients. Possible implications of these cells in the pathogenesis of myasthenia gravis are discussed.

INTRODUCTION

Myasthenia gravis (MG) is a neuromuscular disorder that seems to be caused by a post-synaptic disturbance. During the last few years it has become evident that the basic defect in MG is a reduction of the available acetylcholine receptor (AChR) at neuromuscular junctions (Fambrough, Drachman & Satya-murti, 1973; Drachman *et al.*, 1976; Green, Miledi & Vincent, 1975; Albuquerque *et al.*, 1976). An autoimmune response to AChR appears to be responsible for the impairment of neuromuscular transmission in patients suffering from MG and animals immunized with purified AChR (Lindstrom *et al.*, 1976a, b; Almon & Appel, 1976; Lindstrom *et al.*, 1976c). Moreover, the possibility that cell-mediated immune responses are also involved is suggested by several lines of evidence: blast transformation of lymphocytes from MG patients when incubated in presence of AChRs, and an abnormal autoreactivity in mixed lymphocyte–thymocyte cultures in some patients, have been reported (Richman, Patrick & Arnason, 1976; Abramsky *et al.*, 1975; Abdou *et al.*, 1974). In addition, a more complex defect in immunity is suspected on the basis of the association of MG with some putative autoimmune diseases (Simpson, Behan & Dick, 1976; Simpson, 1960; Oosterhuis & de Hass, 1968; Namba, Brunner & Grob, 1973; Wolf *et al.*, 1966), with thymic pathology (Castleman, 1966; Namba, Nakata & Grob, 1976), a selective IgA deficiency (Simpson, *et al.*, 1976; Bramis *et al.*, 1977), and an increased frequency of extrathymic tumours (Papatestas *et al.*, 1976). The immunological abnormalities in myasthenia gravis might be associated with a change in T lymphocyte subpopulations regulating or suppressing immune responses.

Recently, human T lymphocytes have been shown to express receptors that are able to bind the Fc portion of IgG (T_γ) or IgM (T_μ) (Dickler, Adkinson & Terry, 1974; Ferrarini *et al.*, 1975; Moretta *et al.*, 1975; McConnell & Hurd, 1976). These two subpopulations were shown to have morphological and histochemical characteristics (Grossi *et al.*, 1978). Moreover, Moretta *et al.* (1977) have demonstrated that T_μ lymphocytes act as helpers for the polyclonal B cells' differentiation to plasma cells induced by pokeweed mitogen, whereas T_γ lymphocytes, activated by the interaction with immune complexes, act as suppressors. Recently, high proportions of peripheral T_γ lymphocytes were observed in some patients with immunodeficiency diseases (Moretta *et al.*, 1977; Gupta & Good, 1977) and with multiple sclerosis (Santoli *et al.*, 1978).

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In this paper we report preliminary results of a study concerning the distribution of T cell subsets in MG patients.

MATERIALS AND METHODS

Myasthenia gravis (MG) patients. Twenty-three patients with clinically and electrophysiologically proven MG were studied. The severity of their disease, according to the Osserman clinical classification (Osserman, 1958), and thymus pathology are shown in Table 1. All thymectomized patients were studied before surgery. Only two patients were receiving steroids; the rest were either receiving anti-cholinesterase drugs or were receiving no medication. Age-matched controls consisted of healthy volunteers.

TABLE 1. T lymphocyte subpopulations in myasthenia gravis patients

Patients	Disease stage*	Age/sex	Total			Thymus pathology
			T cells†	T γ	T μ	
B.C.	IIA	54/M	71	33	40	Involuted thymus
C.G.	III	58/M	67	8	58	Involuted thymus
A.C.	IIA	21/F	77	46	21	Involuted thymus
M.M.	I	52/M	64	40	42	Involuted thymus
C.S.	IIA	65/F	76	10	67	Involuted thymus
G.I.	IIA	67/M	78	21	59	Involuted thymus
R.F.	IV	25/F	65	19	47	Hyperplasia
P.M.	IIA	39/F	75	9	47	Hyperplasia
P.C.	IIB	12/F	70	15	59	Hyperplasia
B.A.	IIB	42/F	67	11	68	Hyperplasia
T.N.	IIA	35/F	59	29	46	Hyperplasia
D.F.M.	I	52/M	68	7	49	Hyperplasia
P.N.	IIA	53/M	62	27	51	Thymoma
M.U.	IIB	64/M	80	9	36	Thymoma
A.R.	I	44/F	76	26	60	Thymoma
M.A.	III	39/F	84	10	50	Thymoma
M.G.	IIA	25/M	58	6	44	Thymoma
D.A.	IIA	16/F	73	11	47	Thymoma
I.D.	IIA	57/M	65	7	62	n.d.‡
T.W.	IIB	55/M	76	6	62	n.d.
G.F.	IIB	45/F	55	13	56	n.d.
G.M.	III	53/F	69	11	43	n.d.
S.P.	III	25/M	79	26	25	n.d.

* Disease stage according to the Osserman clinical classification (Osserman, 1958).

† Total T cells are expressed as a percentage of lymphocytes; T γ and T μ cells as a percentage of T cells.

‡ The patient was not thymectomized.

Purification of peripheral T lymphocytes. Peripheral blood mononuclear cells were isolated on a Ficoll-Hypaque density gradient (Böyum, 1968), and adherent cells were removed by incubation at 37°C for 60 min in plastic petri dishes. Lymphocytes were then adjusted to a concentration of 5×10^6 /ml in RPMI 1640 medium with 20% foetal calf serum (FCS) (Flow Laboratories). T lymphocytes were purified from non-T cells by rosetting with neuraminidase-treated sheep erythrocytes (SRBC); 1.0 mg of lyophilized neuraminidase (Sigma Chemical Company, St. Louis) dissolved in 1.0 ml of Hanks's balanced salt solution (HBSS) was added to 10 ml of a 5% SRBC suspension in HBSS. After incubation for 30 min at 37°C, SRBC were washed three times and the final concentration was adjusted to 2% in RPMI 1640 medium containing 20% FCS. 1.0 ml of the lymphocyte suspension (5×10^6 cells) was mixed with an equal volume of 2% SRBC. The mixture was then incubated for 15 min at 37°C and centrifuged at 200 g for 10 min, followed by incubation at 4°C for 60 min. The pellet was gently resuspended and the rosette-forming cells (SE-RFCs) were counted to determine the percentage of T cells in the whole lymphocyte preparation. At least 300 lymphocytes were scored. All lymphocytes binding three or more red blood cells were considered as rosette-forming cells. The SE-RFCs were then separated from the non-rosetting fraction on a Ficoll-Hypaque density gradient. The pelleted SE-RFCs were resuspended and centrifuged again on another density gradient. This procedure gave a final suspension containing more than 96% SE-RFCs. SRBC attached to T cells were lysed by using Tris buffer with

0.83% ammonium chloride. T lymphocytes were then cultured overnight in medium 199 with 20% FCS in a humidified incubator with 5% CO₂, according to the procedure of Moretta *et al.* (1975). At the end of the incubation period, the cells were washed and resuspended to a concentration of 3×10^6 /ml in the same medium.

Preparation of antibody-coated OxRBC (EA). A rabbit antiserum containing mainly IgM anti-OxRBC antibody was raised in rabbits according to Mayer (1961). A purified IgM fraction was obtained by Sephadex G200 gel filtration. An antiserum against OxRBC was raised in rabbits by multiple intravenous injections of washed OxRBC; IgG fraction was purified by DE 52 (Whatman Ltd., Springfield Mill, England) ion-exchange chromatography. Both IgM and IgG anti-OxRBC preparations gave a single precipitation line in immunoelectrophoresis and radial immunodiffusion against rabbit anti-immunoglobulin antisera. Antibody activity was determined by complement-dependent haemolysis; both preparations were then adjusted to a haemolytic titre of 1/512. Sensitization of the erythrocytes with antibodies was performed with IgM and IgG anti-OxRBC, diluted 1:20 and 1:50, respectively. Equal volumes of OxRBC (Dickler, Adkinson & Terry, 1974) in HBSS and diluted IgM and IgG antibody were mixed and incubated at room temperature for 45 min. After washing four times, sensitized OxRBC were suspended in medium 199 containing 20% FCS to give a 1% concentration.

Detection of T cells with receptors for IgM (T μ). 100 μ l of T lymphocyte suspension (3×10^6 /ml) were mixed with 100 μ l of EA-IgM and centrifuged at 200 g for 5 min at 4°C followed by incubation at 4°C for 60 min. The pellet was gently resuspended and 300 lymphocytes were counted for rosette formation. Only lymphocytes with three or more red cells attached were considered as rosette-forming cells.

Detection of T cells with receptors for IgG (T γ). 100 μ l of T lymphocyte suspension were mixed with 100 μ l of EA-IgG. The mixture was centrifuged at 200 g for 5 min at 4°C. The pellet was resuspended and EA (IgG)-RFCs counted immediately.

RESULTS

The percentage of total T peripheral blood lymphocyte and T cell subpopulations in twenty-three MG patients are shown in Table 1. Severity of disease, according to the criteria of Osserman (1958), and thymus pathology, when thymectomy was performed, are indicated in the same Table.

The percentage of circulating T cells was not significantly different in the myasthenic patients compared to the control group (statistical comparisons were performed using the Student's *t*-test). The percentages of T γ and T μ cells in controls were in the range of 5–21 and 36–69, respectively; the mean values were 11.2 for T γ and 53.0 for T μ .

All control subjects had less than 15% T γ cells; only one subject, apparently in good health, exhibited a value of 21%. A substantial increase (>25%) of T γ lymphocytes was observed in seven of the twenty-three myasthenic patients, while in two cases only a slight increase was noted. Two patients showed reduced percentages of T μ cells and both had a concomitant increase of T γ cells.

From our data, there would not appear to be any relationship between disturbances in the balance of T cell subsets, the severity of disease and thymus pathology.

DISCUSSION

Immunological abnormalities in myasthenia gravis have been the focus of a number of studies undertaken to clarify the pathogenesis of this disorder. Many investigators have reported data on the relative distribution of B and T cells in peripheral blood from MG patients.

In this paper, to our knowledge the first concerning the distribution of T γ and T μ cell subsets in myasthenia gravis, we produce evidence for an increased percentage of T γ cells in several patients. Our results indicate that there was no difference in the mean percentage of total T cells, as estimated by the E-neuraminidase-rosette technique, in the peripheral blood of controls as compared to patients with myasthenia gravis, suggesting that myasthenia does not have an effect on the distribution of these cells in the peripheral blood. Similar findings have been reported by other authors (Koziner & Bloch, 1976; Abdou *et al.*, 1974; Kalden *et al.*, 1976; Lisak, Abdou & Zweiman, 1976).

The significance of reduced levels of T μ cells in two patients, both with concomitant T γ increases, is unclear. It is difficult, in the first place, to define what T μ values we can consider to be abnormal, owing to the wide range usually found in healthy controls. A reduction in the proportion of T μ cells, often associated with an increase in T γ cells, has been observed in patients with a variety of immunodeficiency diseases (Moretta *et al.*, 1976; Gupta & Good, 1977). It has been shown that circulating T γ lymphocytes may be present in increased proportions in individuals with thymoma and hypogamma-

globulinaemia, chronic fungal infection and, occasionally, in patients with IgG deficiency, X-linked agammaglobulinaemia and common variable hypogammaglobulinaemia (Moretta *et al.*, 1976; Gupta & Good, 1977). Enhanced suppressor activity demonstrated in most of these diseases could be dependent on an increase in T γ cells. In addition, increased percentages of T γ cells have been demonstrated in cord blood where suppressor activity was also noted (Oldstone, Tishon & Moretta, 1977).

Whilst the exact pathogenesis of myasthenia gravis is unknown, many authors have emphasized the immunodeficiency aspects of this disorder (Simpson *et al.*, 1976; Simpson, 1960). However, an enhanced suppressor activity, possibly supported by the increase in T γ cells that we have shown, has not yet been reported in MG patients. Santoli *et al.* (1978) have recently found an overabundance of circulating T γ cells in multiple sclerosis patients where enhanced immune suppression has not been demonstrated.

The possibility that an antigenic stimulation, mainly in viral infections, may increase the percentage of circulating T γ lymphocytes has been suggested by Santoli *et al.* (1978). Persistent viral infection of the thymus gland in myasthenia was originally hypothesized by Datta & Schwartz (1974). More recently, Tindall *et al.* (1978) have found elevated titres of complement-fixing antibody to cytomegalovirus in MG patients. Therefore, the possibility that increased T γ cell counts might be related to an infectious process cannot be ruled out.

In MG patients, we have not found a relationship between an imbalance of T cell subpopulations and the severity of disease or thymus pathology. However, owing to the limited number of cases studied, further investigations involving larger numbers of patients are needed in order to draw firm conclusions.

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