Lymphocyte subpopulations and surface membrane immunoglobulins in myasthenia gravis

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SUMMARY Lymphocyte subpopulations were studied in 16 myasthenic patients by means of E and EA₇₈ rosettes and surface membrane immunofluorescence. Myasthenic patients showed an increase in lymphocytes with surface IgM and IgD when compared to a control group. In female patients this increase was highly significant, and lymphocytes bearing membrane IgG and IgA were also augmented. No difference was found in male patients when compared with sex-matched controls. These results suggest an activation of the B cell compartment restricted to female patients, and support the hypothesis of different immunopathogenetic mechanisms in this disease.

It is thought that immune mechanisms play an important role in the pathogenesis of myasthenia gravis.¹⁻³ A humoral autoimmune response to acetylcholine receptor impairs neuromuscular transmission and causes clinical symptoms.^{4,5} Cell-mediated immunity seems also to be involved in the pathogenesis of the disease, as suggested by the activation of lymphocytes from myasthenia gravis patients when incubated with acetylcholine receptor,⁶ and by an excessive autoreactivity in mixed lymphocyte-thymocyte cultures in some patients.^{7,8} The role of these two immunological subsets in the pathogenesis of myasthenia gravis is still undefined. Evaluation of T- and B-cell subpopulations resulted in contradictory evidence.⁹⁻¹¹ We present the results of a study concerning lymphocyte subpopulations in myasthenia gravis patients, with particular reference to the immunofluorescence study of surface membrane immunoglobulins (SM Ig).

Patients and methods

Patients Sixteen consecutively hospitalised myasthenic patients were studied. None of them was being treated with corticosteroids or immunosuppressant drugs at the time of study. In each case the diagnosis was established on clinical, pharmacological (edrophonium test) and electrophysiological grounds. Clinical data concerning

the patients are summarised in table 1. The age-matched control population consisted of 64 healthy subjects (32 males and 32 females).

Table 1 Myasthenic patients: clinical data

	Total number (n = 16)	Males (n = 9)	Females (n = 7)
Age*	40.2 ± 20.6	49.1 ± 15.2	28.7 ± 21.8
Time from onset* (years)	5.4 ± 5.2	5.6 ± 5.0	5.2 ± 5.8
Class (according to Osseman)	I : n = 4	n = 2	n = 2
	II : n = 12	n = 7	n = 5
Thymectomised patients	n = 9	n = 5	n = 4

*Results expressed as mean ± 1 SD.

Lymphocyte preparation Lymphocytes were collected from peripheral venous blood according to Böyum.¹²

E-Rosettes This test was performed according to Aiuti *et al.*¹³ Lymphocytes were mixed at the ratio 1:100 with sheep red blood cells (SRBC) resuspended in Hanks' balanced salt solution (HBSS) with 20% of foetal calf serum (FCS; Eurobio, Paris) previously heat-inactivated and adsorbed with SRBC. After incubation for ten minutes at 37°C, the mixture was centrifuged for 5 minutes at 200 g and then kept at 4°C overnight. Each test was carried out in triplicate.

EA₇₈-Rosettes O human red cells (HRBC) were washed three times in phosphate buffered saline (PBS) at 4°C and sensitised with a subagglutinating dose of an IgG fraction obtained by ion-exchange chromatography¹⁴ from a rabbit antiserum against HRBC (Cappel, Cochranville, USA). Briefly, the suspension of 1% HRBC in PBS was mixed (v/v) with a subagglutinating dilution of the antiserum and rotated for 45 minutes at room temperature. Sensitised cells were then washed three times in

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HBSS with 20% of FCS, previously heat-inactivated and absorbed with HRBC. 0.1 ml of the lymphocyte suspension (2×10^6 cells/ml) was mixed with 0.1 ml of the HRBC suspension (10^8 cells/ml), spun at 200 *g* for 5 minutes, and kept at room temperature for 30 minutes. Control tests with unsensitised HRBC were carried out simultaneously.

Surface membrane immunoglobulins (SM Ig) The technique of Loor *et al*¹⁵ was used. Appropriate dilutions of the FITC conjugated antisera (anti-total Ig, anti- γ , anti- μ , anti- α , anti- δ ; Coppel, Cochranville, USA) were determined by direct immunofluorescence staining of normal peripheral blood lymphocytes. After 1 hour incubation in culture medium (Tc199, Eurobio Paris, 10 mM Hepes containing 20% heat-inactivated FCS, glutamine 1% and antibiotics) at 37°C in humidified air containing 5% CO₂, staining was carried out by mixing and incubating 5×10^6 cells with 50 μ l of diluted antiserum in an ice-bath for 30 minutes. Lymphocytes were then washed three times in cold PBS containing 2% bovine serum albumin (BSA, Sigma Chemicals, St Louis, USA) and 0.1% NaN₃ at 4°C. The percentage of positive cells was assessed under fluorescence epillumination with an Orthoplan Leitz microscope. At least 200 cells were scored.

Statistical evaluation was performed using variance analysis.

Results

When the total myasthenia gravis population was compared with the control population no differences could be found either in total lymphocyte counts and percentage of E and EA_{7S} rosettes forming

cells, or in the percentage of lymphocytes with positive surface staining for total immunoglobulins, IgG and IgA. Myasthenia gravis patients showed an increase ($p < 0.025$) in the percentage of lymphocytes bearing membrane IgM and IgD (table 2).

When female patients were compared with female controls, this increase was highly significant ($p < 0.005$), and lymphocytes bearing membrane IgG and IgA also were increased ($p < 0.05$) (table 3). No difference was found between male patients and controls in any of the performed tests. No differences were found when thymectomised patients were compared with non-thymectomised patients, and both groups were compared with controls.

Discussion

Studies of lymphocyte subpopulations in myasthenia gravis patients have yielded conflicting results, mainly concerning T lymphocytes.^{9 10 16-18} Our data confirm the findings of some authors,¹⁶⁻¹⁹ who found no difference between patients and controls. Owing perhaps to the small number of our patients, we did not observe a decrease in T lymphocytes of thymectomised patients, as reported by others.^{20 21} We performed B lymphocyte counts by means of EA_{7S} rosettes and membrane immunofluorescence. This latter technique selectively reveals peripheral blood B cells by detecting endogenous IgM and IgD on the surface membrane, where they act as receptors.^{22 23}

Some authors^{9 16 17 19 24} performed B cell count

Table 2 Lymphocyte subpopulations and surface Ig-lymphocytes*: myasthenia gravis patients versus controls

	Total SM Ig(%)	SM IgM(%)	SM IgD(%)	SM IgG(%)	SM IgA(%)	E-Rosettes (%)	EA _{7S} -Rosettes (%)
Myasthenia gravis patients (n = 16)	20.1 $\pm$ 9.1	10.2 $\pm$ 8.5	7.0 $\pm$ 5.3	9.4 $\pm$ 4.8	3.4 $\pm$ 3.1	57.7 $\pm$ 11.3	13.1 $\pm$ 6.1
Controls (n = 64)	19.9 $\pm$ 3.1	6.4 $\pm$ 1.6	4.1 $\pm$ 1.6	8.7 $\pm$ 2.2	3.1 $\pm$ 1.4	57.6 $\pm$ 8.2	12.3 $\pm$ 2.7
Statistical significance	NS	p < 0.025	p < 0.025	NS	NS	NS	NS

*Results expressed as mean percentage of total lymphocyte counts, $\pm$ 1 SD.
NS = not significant ($p > 0.05$).

Table 3 Lymphocyte subpopulations and surface Ig-lymphocytes*: male and female patients versus controls

	Total SM Ig(%)	SM IgM(%)	SM IgD(%)	SM IgG(%)	SM IgA(%)	E-Rosettes (%)	EA _{7S} -Rosettes (%)
Myasthenia gravis Patients: females	22.0 $\pm$ 11.0	14.5 $\pm$ 10.1	9.8 $\pm$ 6.7	12.2 $\pm$ 6.1	5.2 $\pm$ 3.5	56.2 $\pm$ 11.0	11.1 $\pm$ 6.6
Female controls	20.0 $\pm$ 2.1	6.3 $\pm$ 1.5	4.5 $\pm$ 1.5	8.8 $\pm$ 2.3	3.0 $\pm$ 1.6	57.7 $\pm$ 8.1	12.1 $\pm$ 2.9
Myasthenia gravis Patients: males	18.6 $\pm$ 6.6	7.4 $\pm$ 5.2	4.7 $\pm$ 2.5	7.2 $\pm$ 1.8	2.5 $\pm$ 2.1	58.8 $\pm$ 12.0	15.0 $\pm$ 5.3
Male controls	19.9 $\pm$ 3.0	6.4 $\pm$ 1.7	3.7 $\pm$ 1.6	8.6 $\pm$ 2.2	3.1 $\pm$ 1.2	57.5 $\pm$ 8.6	12.5 $\pm$ 2.4
Female patients versus Female controls	NS	p < 0.005	p < 0.005	p < 0.05	p < 0.05	NS	NS
Male patients versus Male Controls	NS	NS	NS	NS	NS	NS	NS

*Results expressed as mean percentage of total lymphocyte counts, $\pm$ 1 SD.
NS = not significant ($p > 0.05$).

by means of total membrane immunofluorescence, finding no evidence of differences between myasthenia gravis patients and controls. Our results agree with these findings; however, using immune sera with specificity for single immunoglobulin subclasses, we detected an increase of lymphocytes bearing membrane IgM and IgD in myasthenia gravis patients. This difference was highly significant in female patients, who also showed an increase of IgG and IgA bearing lymphocytes. This may be explained by the presence of immune complexes or autoantibodies adhering to the cell membranes,²⁵ pointing to an activation of humoral immunity, as reported in other autoimmune diseases.^{26 27}

None of the performed tests revealed a difference between male patients and sex-matched controls. Thus, the difference found in the myasthenic group as a whole when compared to controls is due to an increase of B cells (IgM and IgD bearing lymphocytes), which is limited to female patients. There is evidence from the literature that myasthenic patients may be divided into two main groups, consisting respectively of young female and older male patients.^{19 28 29} The mean age of our female patients was actually lower than that of males; clinical and immunogenetical studies led some authors to postulate that myasthenia gravis may be due to different mechanisms of breakdown in immunological tolerance.^{30 31} Our findings, pointing to an activation of the B cell compartment restricted to female patients, support the hypothesis of at least two different immunopathogenetic processes in myasthenia gravis.

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