Myasthenia Gravis Three Years after Thymectomy: Immunological Studies*

Izhar Zahavi, M.D.
Luise Komlos, Ph.D.
Zvi Malik, Ph.D.
Phima Shaked, M.Sc.
Carlos Rudniki, M.D.
Isaac Halbrecht, M.D.

Myasthenia gravis is a neuromuscular disorder probably of immune origin which affects acetylcholine receptors at the neuromuscular junction.[1,2] Approximately one tenth of the patients with myasthenia gravis are found to have a thymoma.[3] Diseases which are found to be associated with thymoma and myasthenia gravis are pancytopenia (4%), red cell hypoplasia (4%), and polymyositis (4%).[3] Cellular and humoral immunological abnormalities are common in myasthenia gravis and in 87 per cent of the patients antiacetylcholine receptor antibodies were described.[1] Antithyroid, antinuclear, antilymphocyte antibodies and antibodies to striated muscles that cross react with thymic epithelial cells which contain acetylcholine receptors have also been described in myasthenia gravis.[1,2,4,5] Of particular interest are patients who develop myasthenia gravis after total thymectomy for removal of thymoma. The onset of myasthenia gravis in these patients in the absence of the thymus suggests that the role of the thymus in the disease may be indirect and initiated prior to thymectomy.[3]

SUMMARY   A 43-year-old woman was hospitalised for evaluation of myasthenia gravis which developed three years after thymectomy for thymoma. After surgery the patient was completely asymptomatic for three years. On admission symptoms of myasthenia gravis confirmed by a significant increase in vital capacity after Tensilon test, and antibodies against acetylcholine receptors were found. Immunological studies started at this time revealed an increased proportion of T-active lymphocytes, a decreased proportion of theophylline-sensitive T-suppressor cells and a hyporeactivity of T-suppressor cells. T-lymphocytotoxic and nonlymphocytotoxic IgG-rich fractions which differ by their IgG subclass distribution could be separated from the patient's serum. The lymphocytotoxic fractions were inhibitory to E-rosette formation, to reactive blastogenesis, to mitogen-stimulated proliferation and for the expression of theophylline sensitive receptors on suppressor T cells of normal human lymphocytes. Our data support the suggestion that active subclass specific antilymphocytotoxic antibodies and alterations in the normal level and function of specific T lymphocytes may contribute to maintain the mechanism of autoimmune reactions in myasthenia gravis years after thymectomy.

We report here immunological findings in a patient who developed myasthenia gravis three years after thymectomy. An attempt was made to determine whether alterations in the patient's T lymphocytes could be found and whether the patient's serum could affect immunological reactions of autologous as well as homologous T lymphocytes from healthy controls. A possible correlation between unbalanced T lymphocyte subpopulations and lymphocytotoxic antibodies was also considered.

CLINICAL DESCRIPTION

A 43-year-old woman, mother of 4 children, was admitted to the hospital for evaluation of myasthenia gravis which developed after total thymectomy for thymoma three years previously. Her history dated since ten years ago when she was hospitalised (first admission) because of fever up to 39°C, general malaise, polyarthralgia, myalgia and severe weakness of proximal muscles of shoulders and limb girdles. Routine laboratory tests on admission were normal. Repeated tests for antinuclear antibodies and LE cells were negative. The sedimentation rate was 31 mm/h, SGOT was 51 U (normal 15-45 U), and the creatine phosphokinase (CPK) 16 U (normal 1-3 U). The urinary creatine excretion was elevated – 315 mg/24 h. Electromyography and ECG were normal. Structural changes and atrophic muscle fibres were found in a muscle biopsy. Acute polymyositis was diagnosed. Rapid improvement was obtained after corticosteroid treatment which was gradually discontinued. The patient remained well for four years when she was hospitalised again (second admission). At this time she was pregnant and complained of pains in the shoulders and the upper back. Physical examination revealed muscle tenderness in the shoulders and arms. Laboratory tests including SGOT, SGPT, CPK and urinary creatine were normal. The muscle pains subsided without treatment after a few days and pregnancy evolution and delivery were normal.

The patient was asymptomatic for three more years when she was hospitalised for her third admission with myalgia, swelling of fingers and generalised muscle weakness. The face and eyelids were spared. The patient was unable by herself to rise from supine position. On admission her temperature was 37°C and blood pressure 170/80 torr. The thyroid gland was not palpable. A marked weakness and severe pain of the posterior cervical, and of the shoulder and limb girdle muscles were found. No weakness of ocular muscles and no drooping of eyelids could be observed. A Tensilon test was negative at this time. Normal values were obtained for total leukocyte and differential count, serum urea and creatinine. The sedimentation rate was 43 mm/ h. Elevated values of SGOT – 69 U, SGPT – 63 U, CPK – 6.3 U, and urinary creatine – 500 mg/24 h, were found. The patient was treated with steroids without any improvement. Chest radiography and tomography revealed a lobular mass with radiologic evidence of small calcification in the anterior mediastinum. The patient was operated upon and an encapsulated tumour measuring 4x4x8 cm adherent to the pericardium was found. Microscopically, the tumour was a thymoma which consisted of lymphocytes and epithelial cells and a few Hassal's corpuscles. The tumour cells were found in lobules separated by connective tissue septa.

The patient's condition improved dramatically after the operation and for three years she was asymptomatic till she was again hospitalised (fourth admission) with a history of two weeks of low grade fever and generalised muscle weakness including the eyelids. Nasal voice and dysphagia appeared at the same time. The patient was not able to sit up in bed by herself or to raise her arms. An ECG showed inverted T waves in the precordial leads. A chest radiograph was normal. A Tensilon test found the vital capacity increased from 2,000 to 2,900 ml. Laboratory values including total and differential leukocyte count, blood sugar, urea, uric acid, creatinine, serum proteins, immunoglobulins, complement components C3, C4 were within normal ranges. Rheumatoid factor was negative. SGOT and SGPT were normal. The erythrocyte sedimentation rate was 60 mm/h. Elevated values for CPK – 7.5 U (normal 1-3 U) and aldolase – 70 U (normal 25 U) were found. Urinary creatine was elevated (350 mg/ 24 h). The serum antibody titre against striated muscles was 1:1280 and negative for antinuclear antibodies. The antibody concentration against acetylcholine receptors in the patient's serum was 17.8 pmole alpha bungalotoxin /ml (normal 4.6). The diagnosis of myasthenia gravis was made accordingly.

Administration of prednisone 60 mg and pyridostigmine bromide (Mestinon®) 180 mg daily was started. The Mestinon dose was gradually increased to 300 mg/day while that of steroids was simultaneously reduced.

On follow-up the patient was found to be in good condition for the last four years. She is receiving prednisone 20 mg on alternate days and pyridostigmine bromide 60 mg/day.

IMMUNOLOGICAL TESTS

On the patient's last admission and before any treatment was started, her immune competence was evaluated. For some tests blood was obtained at three different times in the course of 10 days. Blood mononuclear cells were isolated from heparinised blood by the standard Ficoll-Hypaque technique.[6] Serum was stored frozen at -70°C.

Cellular immunity. B and T cell quantitation was performed by the standard immunoglobulin and E-rosette techniques[7] respectively. Active E-rosettes which were reported to be a subset of T cells actively involved in cellular immunity[8-10] were tested according to the method of Wybran and Fudenberg.[10] T suppressor cells were evaluated in two ways: by treatment with theophylline, which was reported to change the E-rosetting capacity of normal lymphocytes and to divide T cells into helper and

suppressor cells,[11,12] and by concanavalin A (con A) activation of T suppressor cells.[13] The patient's con A activated T suppressor cell function was tested on homologous T cell targets stimulated by phytohaemagglutinin (PHA, Wellcome) and con A (Miles, Yeda). The unsuppressed proliferative response to mitogens, PHA, con A, pokeweed (POK — Grand Island) was tested by standard techniques.[14,15] To establish a possible immune reactivity of the patient's lymphocytes to autochtonous cells, lymphocyte reactive blastogenesis was tested.[16]

Effect of the patient's serum and IgG-rich serum fraction on lymphocytes. Serum fractionation was performed by gel filtration on a Sephadex G 100 column.[17] The protein content, the immunoglobulin distribution of the effluents and the electrophoretic mobilities of the IgG-rich fractions were determined by standard methods.[18,19] Titration of IgG subclasses (IgG$_1$, IgG$_2$, IgG$_3$) was performed with subclass specific IgG heavy chain antiserum[20] (The Central Laboratory of the Netherlands Red Cross Blood Transfusion Service). For further characterisation of the IgG-rich column eluted fractions, isoelectric focusing on polyacrylamide gel (Ampholine PAG plates LKB, pH 3.5-9.5) was performed.[21]

To test by trypan blue dye exclusion the cytotoxic or noncytotoxic effect of the patient's whole or absorbed serum,[22] or IgG rich serum fraction on homologous healthy lymphocytes, 0.5 ml fluid were incubated on 8×10^6 cells for 1 hour at 37°C. For some experiments T and B lymphocytes separated by E-rosetting were used. The same incubation procedure was used for the detection of specific IgG or IgM antilymphocytic antibodies with immunofluorescent techniques as well as for E-rosette inhibition tests. To test the effect of the patient's serum on mitogen stimulated lymphocyte cultures, 20% serum was present in each culture.

RESULTS

The percentage of the patient's B and total T lymphocytes was normal (Table 1). The patient's T active lymphocytes were elevated (Table 1). On repeated examinations no theophylline sensitive T suppressor lymphocytes could be detected. The patient's con A

Table 1 Evaluation of the patient's immunocompetence

Lymphocyte source	B-lymphocytes					T-lymphocytes	
	EAC rosettes	Surface immunoglobulin,%				Rosettes, %	
		IgG	IgM	IgA		E	E active
Patient*	24 ± 3	6 ± 0.5	8 ± 0.6	2 ± 0.2		66 ± 6	33 ± 1
Controls**	21 ± 3	7 ± 0.4	8 ± 0.2	2 ± 0.1		61 ± 6	18 ± 2

EAC rosettes: Erythrocyte-antibody-complement rosette method
Surface immunoglobulins: Immunofluorescent technique
*Mean values ± SD for 3 examinations
**Mean values ± SD for 8 healthy controls

Table 2 Evaluation of T suppressor lymphocytes: effect of serum

Lymphocyte source	Theophylline-sensitive T suppressor lymphocytes, %					T suppressor function on mitogen stimulated lymphocytes (% of Suppression)**	
	Untreated	Whole* serum	Fractions*			PHA	Con A
			8	9	10		
Patient+	5	2 (25)	3 (18)	4 (22)	15 (25)	7	12
Controls#	42	10 (40)	12 (35)	14 (41)	35 (42)	68	77

* Before addition of theophylline (5×10^{-4}M) and E rosette test 1 h incubation with sera or serum fractions. In brackets results for control serum or control serum fractions with equivalent protein and IgG concentrations.

** % of suppression = $1 - \dfrac{\text{Values in the presence of suppressor cells}}{\text{Values in the absence of suppressor cells}} \times 100$

+ Mean values for 3 examinations; SD < 10%
# Mean values from 4 healthy donors; SD < 10%

Table 3 Effect of the patient's serum on mitogen stimulated lymphocytes (PHA, POK) and on reactive blastogenesis (RB)

Lymphocyte source	Serum source (Incorporation of ^{3}H thymidine, 10^3 cpm)					
	Patient			Controls		
	PHA*	POK	RB**	PHA	POK	RB
Patient +	116.7	9.6	2.1	127.3	10.2	2.2
Controls#	28.6 ± 16.2	10.9 ± 1.6	0.8 ± 0.1	61.7 ± 14.1	11.4 ± 2.4	2.6 ± 0.5

* 5 μg/ml PHA or POK, 20% serum 4 days in culture
** For RB 3 hours incubation with 20% serum and 2 μCu ^{3}H_
+ Mean values of triplicates; SD < 10%
# Mean values ± SD for 3 healthy donors.

activated suppressor cells were hyporeactive to PHA and con A stimulated T cells (7% and 12% suppression versus 68% and 77% in controls, Table 2). The unsuppressed patient's lymphocytes had a normal response to PHA and POK mitogen, and their reaction was normal for reactive blastogenesis in autologous and in homologous serum (Table 3).

The patient's serum was highly cytotoxic to normal T lymphocytes (Table 4) and an inhibitory effect on E-rosette formation (Table 4), on theophylline sensitive T suppressor cells (Table 2), on lymphocyte proliferative response and on reactive blastogenesis (Table 3) was observed. Absorption of the patient's serum on normal lymphocytes reduced these effects signifi-

cantly (Table 4).

Immunofluorescent studies revealed that the antilymphocytic antibody in the patient's serum is belonging to the IgG class. Two out of three IgG-rich serum fractions (8 and 9) had similar cytotoxic effects, like the patient's serum, on normal lymphocytes (Tables 4 and 5). The noncytotoxic fraction 10 had no such effects on T cell

Table 4 Effect of the patient's serum and IgG rich serum fractions on control lymphocytes

Serum or fraction	Cytotoxicity assay (%)*			Immunofluorescence (%)**		E- rosettes (%)
	Unseparated lymphocytes	T	B	T cells Anti-IgG	Anti-IgM	
Serum †						
Unabsorbed	46 (4)	61 (11)	7 (9)	64 (3)	3 (2)	4 (60)
Absorbed	17 (7)	ND	ND	ND	ND	45 (61)
IgG-rich eluted serum fraction †						
Tube No. 8	ND	37 (9)	10 (6)	55 (5)	2 (3)	21 (48)
9	ND	31 (11)	8 (9)	52 (3)	4 (2)	13 (58)
10	ND	10 (11)	11 (10)	12 (4)	3 (4)	55 (50)

* % of trypan blue stained cells
** % of immunofluorescent stained cells
† Mean values for lymphocytes from 3 healthy donors; SD < 15%. For comparison, in brackets mean values for control serum or eluted fractions with equivalent protein and IgG concentration.

Table 5 Effect of the patient's IgG-rich serum fractions on mitogen stimulated control lymphocytes

Tube No.	Protein	IgG	Incorporation of ^{3}H thymidine (10^3 cpm)*	
			PHA	POK
8	4.9	2.4	2.2	7.6
	0.5**	0.24 (0.22)***	8.2 (62.6)	8.2 (10.7)†
9	4.1	1.9	2.1	8.3
	0.5	0.23 (0.21)	10.2 (88.2)	7.2 (7.6)
10	3.6	2.0	73.5	8.2
	0.5	0.26 (0.28)	69.2 (71.8)	8.4 (8.1)

* Mean values of lymphocyte cultures from 3 healthy donors; SD < 20%
** Eluted fraction adjusted in PBS to 0.5 mg/ml protein
*** In brackets IgG concentrations of equivalent fractions of control serum. IgM and IgA (test and control) in original elutes from 40-60 µg and 20-40 µg/ml, respectively.
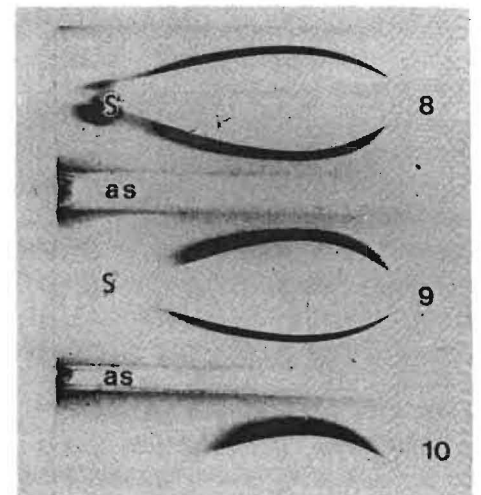† Cultures incubated with control serum fractions.

Fig. 1 Immunoelectrophoretic behaviour of column eluted serum fractions 8, 9, 10 (2.5 mg/ml protein)
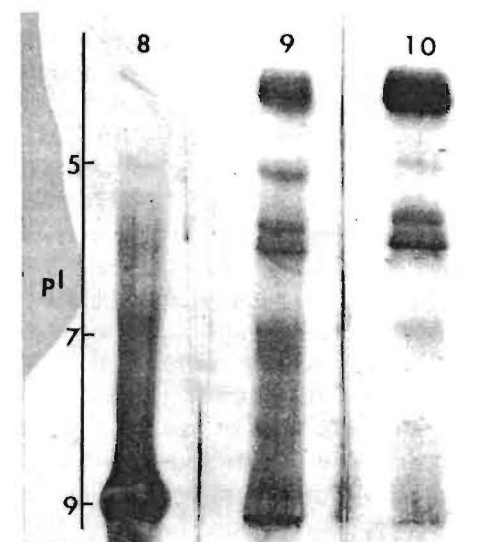S = sample
AS = goat antihuman IgG heavy chain antiserum

Fig. 2 Isoelectric spectra of column eluted serum fractions 8, 9, 10. (2.5 mg/ml protein). PI = isoelectric pH.

Table 6 IgG subclass distribution of the patient's IgG rich eluted serum fractions

Tube No.	IgG_1	IgG_2	IgG_3	IgG_4
8*	1/2	1/16	1/4	1/8
9	–	1/16	1/2	1/8
10	–	1/2	–	1/8

*Samples adjusted to 2.5 mg/ml protein. Results expressed as the lowest dilution at which a precipitin line was observed.

specific reactions (Table 4 and 5). Similar to normal serum, fraction 10 rendered the patient's lymphocytes able to express theophylline sensitive receptors on T suppressor lymphocytes (Table 2). The cytotoxic and noncytotoxic IgG-rich serum fractions differ by their electrophoretic mobilities (Fig. 1), isoelectric spectra (Fig. 2), and IgG subclass distribution (Table 4). Faster electrophoretic mobilities due probably to a higher concentration of IgG_2[23] could be observed in the cytotoxic fractions 8 and 9 (Fig. 1 and Table 6). Lack of IgG_3 could be observed by isoelectric focusing (pH range for IgG_3 8.2-9[21]) and with specific antisera, for the noncytotoxic fraction 10 (Fig. 2 and Table 6).

DISCUSSION

The immunopathogenesis of myasthenia gravis has possibly two mechanisms: the first, well proved, is the humoral antiacetylcholine receptor antibody mechanism and the second, the cell mediated immune mechanism. It is established that antiacetylcholine receptor antibodies of IgG class can bind to acetylcholine receptors in about 80 per cent of patients with myasthenia gravis. However, additional factors produced by the thymus may interact with the antiacetylcholine receptor antibodies in damaging the acetylcholine receptors.[24] Abnormalities are found in the thymus in about 80 per cent of the patients with myasthenia gravis, 65 per cent of whom have thymic hyperplasia and about 10 per cent epithelial or lymphomatous thymoma. The fact

that 60-80 per cent of the patients with myasthenia gravis show partial or complete remission after thymectomy suggests the important role of the thymus in this disease.[24]

The onset of myasthenia gravis in the absence of the thymus raises the question as to whether the alterations in certain of the patient's T cells may be in some way correlated to the patient's thymus prior to thymectomy and whether lymphocytotoxic antibodies found in the patient's serum may be associated with specific T cell subsets.

A correlation between unbalanced lymphocyte populations and distinct types of antibodies were found in autoimmune conditions including myasthenia gravis.[25-28] In the present case the subset of T active lymphocytes was found to be increased as compared to control lymphocytes. Different humoral thymic factors have been shown to influence different T cell subpopulations.[29] Thymosin was found to induce the appearance of T lymphocyte surface markers on T active lymphocytes and to raise the percentage of T active E-rosettes.[8,30-32] Since the life span of some thymus derived lymphocytes is very long, probably several years,[3] it may be suggested that an increased output of thymic hormones, prior to the removal of the thymoma, may have changed surface properties on the patient's T active lymphocytes. Such changes may subsequently contribute to an increase in the density and/or affinity of surface receptors for sheep red blood cells on these lymphocytes.

A decreased level of specific T suppressor cells and a functional

disorder of T suppressor cells could be also demonstrated on the patient's lymphocytes. A correlation between the patient's IgG–rich lymphocytotoxic serum fractions and specific suppressor T lymphocytes could be observed. The lymphocytotoxic IgG-rich fractions 8 and 9 eluted from the patient's serum were inhibitory to theophylline sensitive T suppressor cells. In contrast to this, incubation with the noncytotoxic IgG-rich fraction 10, similar to control serum, rendered the patient's and homologous lymphocytes sensitive to theophylline. As fraction 10 differs from serum fractions 8 and 9 in IgG subclass distribution, the results suggest that an IgG subclass specific lymphocytotoxic antibody is binding to specific T suppressor lymphocytes.

An increased sensitivity of T suppressor lymphocytes to lymphocytotoxic antibodies was observed in some autoimmune conditions, including myasthenia gravis.[5,33] An IgG antibody directed toward theophylline sensitive T suppressor lymphocytes was found in childhood myasthenia gravis.[26] No IgG subclass correlated antibody to T suppressor lymphocytes was, to our knowledge, reported in myasthenia gravis. The demonstration of cellular and humoral immunoregulation abnormalities and its correlation in a patient with myasthenia gravis three years after thymectomy may be of interest and suggests the possible long acting influence of the thymus in the pathogenesis and maintenance of the disease.

REFERENCES

1. Drachman DB. Myasthenia gravis. N Engl J Med 1978; 298:136-42.

2. Hoffman PM. Neurologic diseases. Myasthenia gravis. In: Fudenberg HH, Stites DP, Caldwell JL, Wells JV, eds, Basic and clinical immunology. 2nd ed. Los Altos, California: Lange Med Pub 1978:648-9.

3. Namba T, Brunner N, Grob D. Myasthenia gravis in patients with thymoma, with particular reference to onset after thymectomy. Medicine 1978; 57:411-33.

4. Adner MM, Sherman JD, Ise C, Schwab RS, Dameshek W. An immunologic survey of 48 patients with myasthenia gravis. N Engl J Med 1964; 271:1327-33.

5. Mischak RP, Dan PC. Lymphocyte binding antibodies and suppressor cell activity in myasthenia gravis. Ann NY Acad Sci 1981; 377:436-46.

6. Boyum A. Isolation of mononuclear cells and granulocytes from peripheral blood. Scand J Clin Lab Invest 1968; 21 (Suppl): 77-89.

7. Halbrecht I, Komlos L. E-rosette forming lymphocytes in mother and newborn. Lancet 1976; 1:544.

8. Fudenberg HH, Wybran J, Robbin D. T-rosette forming cells, cellular immunity and cancer. N Engl J Med 1975; 292:475-6.

9. Felsburg PJ, Edelman R, Gilman RH. The active E rosette test: correlation with delayed cutaneous hypersensitivity. J Immunol 1976; 116:1110-4.

10. Wybran J, Fudenberg HH. Thymus-derived rosette forming cells in various human disease states: cancer, lymphoma, bacterial and viral infections, and other diseases. J Clin Invest 1973; 52:1026-32.

11. Limatibul S, Shore A, Dosch HM, Gelfand EW. Theophylline modulation of E-rosette formation: an indicator of T-cell maturation. Clin Exp Immunol 1978; 33: 503-13.

12. Shore A, Dosch HM, Gelfand EW. Induction of antigen dependent T helper and T suppressor cells in man. Nature 1978; 274:586-7.

13. Hubert C, Delespesse G, Govaerts A. Concanavalin A activated suppressor cells in normal human peripheral blood lymphocytes. Clin Exp Immunol 1976; 26:95-8.

14. Halbrecht I, Komlos L, Strauss Z. Lymphocyte reactivity in the aged. J Am Ger Soc 1977; 25:354-7.

15. Komlos L, Halbrecht I, Schachor J, Djaldetti M. The effect of sera from chronic lymphocytic leukemia patients on normal mitogen stimulated lymphocytes. A possible correlation with the patient's T and B lymphocytes. Exp Hematol 1978; 6:595-600.

16. Harris JE, Stewart THM. Recovery of mixed lymphocyte reactivity (MLR) following cancer chemotherapy in man. In: Schwarz MR, ed, Proceedings of the VI Leucocyte Culture Conference. New York: Academic Press, 1972:555.

17. Fahey JL, Terry EW. Ion exchange chromatography and gel filtration. In: Weir DM, ed, Handbook of experimental immunology and immunochemistry. 2nd ed. Oxford: Blackwell Sci Pub 1973.

18. Peterson GL. A simplification of the protein assay method of Lowry et al, which is near generally applicable. Biochemistry 1977; 83:346-56.

19. Mancini G, Carbonara AO, Heremans JF. Immunochemical quantitation of antigen by single radial immunodiffusion. Int J Immunochem 1965; 2:235-54.

20. Ouchterlony O. Diffusion in gel methods for immunological analysis. II. In: Kallos P, Waksmann BH, eds, Progress in allergy VI. Basel: Karger, 1962:30.

21. Williamson AR. Isoelectric focusing of immunoglobulins. In: Weir DM, ed, Handbook of experimental immunology. I: Immunochemistry. 2nd ed. Oxford: Blackwell Sci Pub 1973.

22. Moroz C, Giler S, Kupfer B, Urca I. Ferritin bearing lymphocytes and T cell levels in peripheral blood in patients with breast cancer. Cancer Immunol Immunother 1977; 3:101-5.

23. Virella G, Howard A. Correlation between electrophoretic mobility and heavy chain sub-classes of residual IgG from patients with severe hypogammaglobulinaemia. Experientia 1970; 26:901-3.

24. Juguilon AC, Bradley W. New insights into myasthenia gravis. In: Isselbacher KJ,

Adams RD, Braunwald E, Petersdorf RG, Wilson JD, eds, Harrison's principles of internal medicine, Update III. New York: McGraw-Hill, 1982:223.

25. Koike T, Kubayashi S, Yoshiki T, Itoh T, Shirai T. Erythrocyte rosette inhibition as an assay for natural occurring T lymphocyte antibody in systemic lupus erythematosus. Arthr Rheum 1979; 22:1064-73.

26. Shore A, Limatibul S, Dosch HM, Gelfand EW. Identification of 2 serum components regulating the expression of T-lymphocyte function in childhood myasthenia gravis. N Engl J Med 1979; 301:625-9.

27. Kelley RE, Keesey JC, Goymerac Y, Larrick SB, Kebo D, Buffkin D. Immunoregulation of total IgG synthesis in myasthenia gravis. Ann NY Acad Sci 1981; 377:403-10.

28. Wilcox N, Thomas JA, Granger S, Newsom-Davis J, Janossy G. Immunopathological studies on lymphocyte subpopulations involved in autoantibody production in myasthenia gravis. Ann NY Acad Sci 1981; 377:898.

29. Stites DP, Caldwell JL. Phylogeny and ontogeny of the immune response. In: Fudenberg HH, Stites DP, Caldwell JL, Wells JV, eds, Basic & Clinical immunology. 2nd ed. Los Altos, California: Lange Med Pub 1978:141-53.

30. Scheinberg MA, Cathcart ES, Goldstein AL. Thymosin-induced reduction of "null-cells" in peripheral blood lymphocytes of patients with systemic lupus erythematosus. Lancet 1975; 1:424-9.

31. Dopp AC, Mutchnick MG, Goldstein AL. Thymosin-dependent T-lymphocyte response in inflammatory bowel disease. Gastroenterology 1980; 79:276-82.

32. Chilsholen RL, Tubergen DG. The significance of varying SRBC/lymphocyte ratio in T cell rosette formation. J Immunol 1976; 116:1397-9.

33. Koike T, Kobayashi S, Yoshiki T, Itoh T, Shirai T. Differential sensitivity of functional subsets of T cells to the cytotoxicity of natural T-lymphocytotoxic autoantibody of systemic lupus erythematosus. Arthr Rheum 1979; 22:123-9.