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Evaluation of T cell subsets in myasthenia gravis using anti-T cell monoclonal antibodies

SONIA BERRIH,* C. GAUD,* MARIE-ANNE BACH,† H. LE BRIGAND,* J. P. BINET* & J. F. BACH† * *Centre Chirurgical Marie-Lannelongue, Le Plessis-Robinson, and* † *INSERM U 25, Hôpital Necker, Paris, France*

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SUMMARY

Functional T cell subsets have been evaluated in the peripheral blood of patients with myasthenia gravis using monoclonal anti-T cell antibodies and a suppressor cell assay based on the suppression of the mixed-lymphocyte reaction by concanavalin A-activated lymphocytes. A significant decline of suppressor cells was found in a large proportion of patients, both by direct count using the anti-suppressor-cytotoxic T cell antibody (OKT8) and by the suppressor assay. Patients also showed an increase in immature T cells defined by their simultaneous reaction with the anti-helper cell (OKT4) and anti-suppressor cell (OKT8) antibody. Thymectomy tended to enhance the deficit in suppressor cells, and to induce the disappearance of double-labelled cells.

INTRODUCTION

Patients with myasthenia gravis (MG) present with a number of immunological abnormalities. Their serum contains various autoantibodies, particularly anti-acetylcholine receptor antibodies whose pathogenic role has been strongly suggested by experimental data (Kao & Drachman, 1977; Drachman *et al.*, 1978; Stanley & Drachman, 1978). Their thymus glands are often morphologically abnormal (tissue hyperplasia, or more rarely thymoma), and thymectomy has been reported to improve the clinical state of a large percentage of patients (Papatestas *et al.*, 1971; Le Brigand *et al.*, 1972). Experimentally-induced thymitis leads to a myasthenia-like syndrome in guinea-pigs and thymopoietin, a thymic peptide, induces a neuromuscular block *in vitro* (Goldstein & Manganaro, 1971). Furthermore, MG is significantly associated with the HLA antigen B8 (Feltkamp *et al.*, 1974; Fritze *et al.*, 1974; Terasaki & Milkey, 1975) known to be linked with several autoimmune diseases.

Collectively, these data indicate that MG probably has an immunological origin. Consequently, it would be important to determine the nature of the immunological imbalance which is likely to predispose to the appearance of the immunological mechanisms responsible for the disease.

New methods have become recently available for studying T cell function in man. Various *in vitro* tests allow the evaluation of some T cell functions such as the capacity to proliferate in the presence of mitogens, soluble antigens and alloantigens, or the generation of suppressor or cytotoxic T cells. More recently, monoclonal anti-T cell antibodies have been available. These antibodies were produced by hybridizing spleen cells from mice immunized against human T cells with non-Ig-secreting myeloma cells (Reinherz *et al.*, 1979a). Previous studies demonstrated that these antibodies recognize distinct subsets (Kung *et al.*, 1979; Reinherz *et al.*, 1979b, 1980; Reinherz & Schlossman, 1980).

Correspondence; Sonia Berrih, Centre Chirurgical Marie-Lannelongue, 133 avenue de la Résistance, 92350 Le Plessis-Robinson, France.

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In the peripheral blood, OKT3 antibody reacts with all T cells, OKT4 with inducer T cells, OKT5 and OKT8 with suppressor and cytotoxic T cells. OKT6 and OKT9 antibodies do not recognize peripheral T cells. OKT10 is expressed on 5% of peripheral T cells but is not restricted to cells of T lineage (Reinherz *et al.*, 1980).

In the thymus, three different subsets have been defined: the early thymocyte with OKT9, OKT10 phenotype, the cortical thymocyte which bears the OKT10, OKT6, OKT4, OKT8 and OKT5 antigens and the mature thymocyte which is either $OKT10^+$ $T3^+$ $T4^+$ or $OKT10^+$ $T3^+$ $T8^+$ $T5^+$.

In order to investigate the potential T cell imbalance present in MG patients, we have applied some of the new techniques mentioned above to a series of MG patients studied, in most cases, before and after thymectomy.

PATIENTS AND METHODS

Patients

Myasthenia gravis was defined by the presence of an abnormal muscular fatigability sensitive to anti-acetylcholine esterase treatment. Clinical data are shown in Table 1. The Osserman clinical classification (Osserman, 1958) was used to define the disease stage. All patients were receiving anti-acetylcholine esterase drugs.

Immunofluorescence studies

The mononuclear cell suspension isolated from venous blood on Ficoll–Hypaque gradient was adjusted to 25×10^6 cells/ml. The immunofluorescence test was performed by incubating 45 μl of the cell suspension with 5 μl of monoclonal antibodies at optimal dilution for 30 min at 4°C in HBSS

Table. 1. Clinical data and peripheral T subsets in MG patients before thymectomy

Patients	Sex	Age	Severity: Osserman classification	Duration of disease	Thymus pathology	EN rosettes	$OKT3^+$ cells	$OKT4^+$ cells	$OKT8^+$ cells
AUD	F	53	IIa	3 years	Involuted	75	70	60	11
MAR	F	34	I	1 year	Involuted	85	75	52	25
DAV	M	71	IIa	19 years	Involuted	65	69	46	36
DOU	F	49	IIa	6 months	Involuted	n.t.	81	58	27
PAP	F	18	IIb	6 months	Hyperplasia	76	n.t.	n.t.	n.t.
MAH	M	53	IIa	8 years	Hyperplasia	75	64	45	35
BER	M	23	IIa	3 years	Hyperplasia	62	60	54	14
ELB	F	31	IIa	2 years	Hyperplasia	87	88	68	45
JAO	M	25	I	3 months	Hyperplasia	59	42	27	13
TEM	M	13	IIa	1 year	Hyperplasia	73	76	49	23
ROU	F	56	IIa	8 years	Hyperplasia	80	84	53	26
LAP	M	50	IIa	23 years	Hyperplasia	80	66	62	31
BOU	F	24	IV	7 years	Hyperplasia	78	68	50	10
BOR	M	52	IIa	6 months	Thymoma	85	84	58	42
LEF	F	56	IIb	11 months	Thymoma	82	61	44	32
RAM	M	53	IIa	2 months	Thymoma	77	66	49	14
KEN	F	46	IIa	2 years	Thymoma	79	73	55	22
PON	F	61	IIb	4 months	Thymoma	92	85	48	27
LOO	F	60	IIa	1 year	Thymoma	67	75	55	28
Controls						76·4 ± 1·8	74·9 ± 1·6	50·4 ± 2·1	29·8 ± 1·2

Results of the controls are expressed as the mean ± s.e.m.
EN rosettes represent the rosette test using neuraminidase-treated sheep red blood cells.

supplemented with 5% fetal calf serum and 1% sodium azide. The cells were then washed twice, resuspended in medium and incubated with 5 μl of fluorescein-conjugated goat anti-mouse Ig serum for 30 min at 4°C, and finally washed twice. The pellet was resuspended in a minimal amount of medium and examined in a Leitz Orthoplan microscope equipped for epifluorescence.

In this study, we have used the OKT3, OKT4, OKT6, OKT8 and OKT10 antibodies whose specificities are described above.

The simultaneous presence of OKT4 and OKT8 antibodies on the cell surface has been investigated by comparing the number of fluorescent cells after incubation with OKT4 alone, OKT8 alone and both antibodies. When cells bear simultaneously both markers, the sum of the figures found with the OKT4 and OKT8 antibodies added alone is superior to that found when using the mixture of OKT4 + OKT8 antibodies. The difference defines the number of cells bearing both markers.

E rosette test. The total number of T cells was evaluated by the spontaneous sheep cell rosette test (Bach, 1973). To enhance the rosette stability, the SRBC were neuraminidase-treated as previously described (Galili & Schlesinger, 1974).

Mitogen responses. Lymphocytes (5×10^5) were cultured in Falcon plates (Microtest II), in 180 μl of medium (RPMI 1640 supplemented with 10% fetal calf serum, 1% glutamine and 2% penicillin–streptomycin) with 20 μl of mitogens (phytohaemagglutinin at 2 and 0·5 μg/ml, concanavalin A at 20 and 5 μg/ml). After a 48-hr culture period, 1 μCi ^{3}H-thymidine was added to each well for 20 hr. The cells were then harvested and the ^{3}H-thymidine incorporation was determined by counting the radioactivity on the filters in a liquid-scintillation counter. All cultures were performed in quadruplicate.

Con A-induced suppressive activity. The suppressive activity of Con A-activated peripheral blood mononuclear cells was assessed on MLC, as already described by Sakane & Green (1977).

Statistical tests. The Student *t*-test was used throughout.

RESULTS

T cell subsets, as defined by monoclonal antibodies

Lymphocytes obtained from the peripheral blood of MG patients were successively incubated with the various anti-T cell monoclonal antibodies described above.

OKT3$^+$ cells (total T cells). The OKT3 antibody recognizes most peripheral T cells. The level of OKT3$^+$ cells was not significantly altered in MG patients (Table 1).

OKT4$^+$ cells (inducer T cells). No difference was observed in the percentage of OKT4$^+$ cells in MG patients compared to controls (Table 1).

OKT8$^+$ cells (suppressor/cytotoxic T cells). The average of OKT8$^+$ cells was slightly lower in MG patients than in normal controls (25·6 versus 29·8). This difference is not significant when one considers all tests performed, even if there are clearly some MG patients showing decreased OKT8$^+$ cell percentages (Table 1). However, the difference between MG patients and controls becomes highly significant when one excludes the double-labelled OKT4$^+$ T8$^+$ cells and considers the level of OKT4$^-$ T8$^+$ cells (15·3% in MG patients versus 26·10% in controls, $P < 0{\cdot}001$) (Fig. 1). The incidence of patients with low OKT8$^+$ cells increased after thymectomy (2–4 weeks post-Tx) and the difference with normal controls was then significant both for total OKT8$^+$ cells (22·7 versus 29·8%, $P < 0{\cdot}001$) and for OKT4$^-$ T8 $^+$ cells (16·3% versus 26·1, $P < 0{\cdot}001$).

It is interesting to consider the ratio of OKT4$^+$ T8$^-$/OKT8$^+$ T4$^-$ cells. Fig. 2 shows the results presented this way. One may note that 13 out of 16 non-thymectomized patients, and 14 out of 17 thymectomized patients showed OKT4$^+$ T8$^-$/OKT8$^+$ T4$^-$ ratios greater than the average ratio observed in normal controls.

The number of OKT4$^+$ T8$^+$ cells which was very low in normal subjects (0–5%) was increased in MG patients studied before thymectomy. Interestingly, this number fell to zero in the great majority of cases after thymectomy (Fig. 1). In the antibody-association test used as described above, we have checked that OKT4$^+$ cells and OKT8$^+$ cells also bear the OKT3 antigen.

One may note that the number of OKT6$^+$ cells was null or very low ($< 2\%$) in normal subjects. It

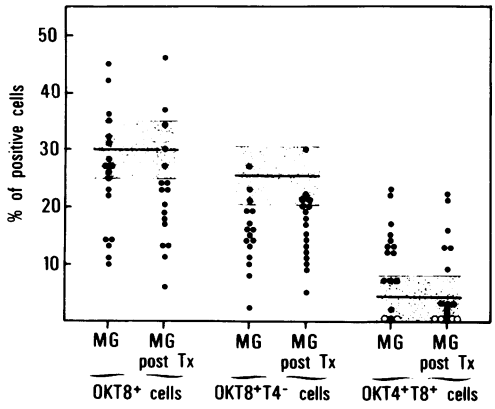

Fig. 1. Percentage of OKT8$^+$, OKT8$^+$ T4$^-$ and OKT8$^+$ T4$^+$ cells in the peripheral blood of MG patients before and 2 weeks after thymectomy. The bar represents the mean ($\pm$s.d.) of the controls. (o) Negative values ($<5\%$). The number of OKT8$^+$ T4$^-$ cells is obtained by subtracting the number of OKT4$^+$ cells from the number of positive cells in the sample with both OKT4 and OKT8 antibodies. The number of OKT8$^+$ T4$^+$ cells is obtained by subtracting the number of positive cells in the sample added with both antibodies from the sum of values found with OKT4 and OKT8 antibodies used alone.

was also very low in MG patients. Levels of OKT10$^+$ cells were also usually low but significant levels of OKT10$^+$ cells were incidentally found in some patients as well as in some control subjects.

Clinical correlation. No difference was observed between the decline of OKT8$^+$ cell number, the presence of double-labelled cells with clinical parameters (duration and severity of the disease, age, sex, presence of a thymoma).

E neuraminidase rosettes

The percentage of E neuraminidase rosette-forming cells was normal in all MG patients studied (average: $76{\cdot}5 \pm 2{\cdot}2\%$) compared to control values ($76{\cdot}4 \pm 1{\cdot}8\%$). Individual results are shown in Table 1.

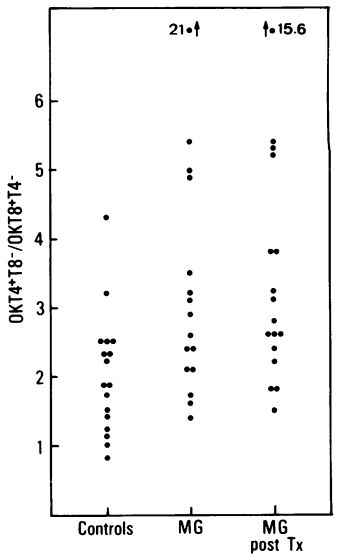

Fig. 2. OKT4$^+$ T8$^-$/OKT8$^+$ T4$^-$ ratio in MG patients before and after thymectomy compared to controls.

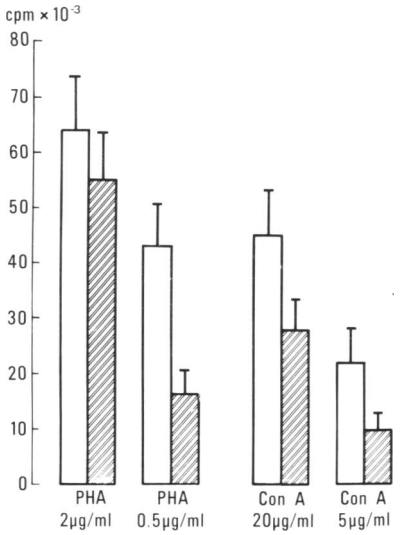

Fig. 3. Mitogen responses of peripheral blood from MG patients to optimal and infra-optimal concentrations of PHA and Con A. Results are expressed as mean ± s.e.m. (□) Controls, (▨) MG.

Lymphocyte proliferative responses

The overall MG lymphocyte response to mitogens was normal when using optimal mitogen concentrations (Fig. 3). There was, however, a tendency towards a decreased response to infra-optimal mitogen concentrations, which was statistically significant for 0·5 μg/ml PHA concentration ($P < 0{\cdot}01$).

Suppressor T cell assays

Normal peripheral blood lymphocytes usually exert intense suppression of autologous MLC after Con A activation with suppression indices between 30 and 80%. Five out of 10 MG patients (without thymoma) showed depressed suppressor function in this assay, while their allogeneic response was normal compared to controls. Conversely, MG patients with thymoma were essentially normal (Fig. 4). No obvious correlation was found between the low suppressor cell

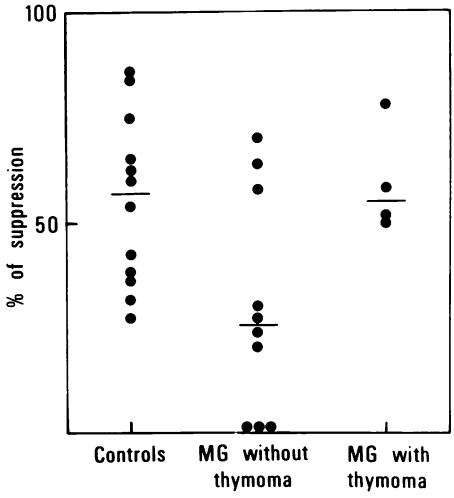

Fig. 4. Evaluation of suppressor T cell function in MG patients with or without thymoma. Suppression index is defined in the Patients and Methods section. Medium values are indicated by a bar.

function defined in the Con A suppression assay and decreased OKT8$^+$ cell values. This functional study has not been performed in thymectomized patients.

DISCUSSION

A number of investigators have attempted to delineate the putative T cell functional anomalies present in MG. Most studies dealt with peripheral blood lymphocytes, a smaller number with thymic lymphocytes. In general, limited abnormalities have been observed. E rosette values and membrane Ig-bearing cells have been found to be essentially normal (Itoyama *et al.*, 1979; Wijermans *et al.*, 1980). Mitogen responses were reported to be normal (Wijermans *et al.*, 1980) or marginally depressed (Huang, Rose & Mayer, 1977; Zilko *et al.*, 1979). The thymus gland is known to be frequently microscopically or macroscopically abnormal (thymoma or hyperplasia) but thymic lymphocytes do not show major functional disturbances (Birnbaum & Tsairis, 1977; Abdou *et al.*, 1974; Opelz *et al.*, 1978; Richman, Patrick & Arnason, 1976). This relative failure to detect clear abnormalities in T cell functions in MG patients is at contrast with the presence of thymic morphological changes and of the multiple immunological abnormalities present in MG, including the existence of various autoantibodies, the appearance of which is generally thought to involve a T cell imbalance. Two major technical breakthroughs have recently appeared in the study of human T cells. The first one relates to the possibility, made recently available, of enumerating the various T cell subsets by the use of specific anti-T cell monoclonal antibodies produced by mouse hybridomas. The other one concerns the capability of analytically studying T cell function *in vitro*. For example, suppressor T cells may be studied after they have been activated by the T cell polyclonal activator, Con A. This study reports the first results obtained by using these two new approaches.

Our results confirm those of previous studies showing the absence of major changes in the number of total T cells in the blood of MG patients, whether using the E rosette test or the OKT3 antibody directed against all peripheral T cells.

The analysis of T cell subsets with the monoclonal antibodies indicates that a fraction of MG patients definitely show a deficit in OKT8$^+$ cells. Not all MG patients show such a deficit, but a minority of them clearly do (five of 18 in our series) when one considers all OKT8$^+$ cells, or even the majority of them, if one considers OKT4$^-$ T8$^+$ cells. The decreased level of OKT8$^+$ cells indicates a deficiency of suppressor T cells, in keeping with the data of the Con A-induced suppressor assay, although one cannot strictly exclude a diminution of cytotoxic T cells, also labelled by the OKT8 antibody. It would be interesting to know if patients showing low OKT8$^+$ cell values also include predominantly patients with HLA-B8, DR3 antigens, as in Zilko's series (Zilko *et al.*, 1979).

The deficit of suppressor T cells is observed in thymectomized patients, but is not clearly accentuated by thymectomy. One should bear in mind, in that regard, the decrease in suppressor T cell function noted in mice after adult thymectomy (Kerbel & Eidinger, 1972; Rotter & Trainin, 1974). It should be noted, in addition, that in the mouse, adult thymectomy induces the loss of Lyt 123$^+$ cells (the putative equivalent of OKT4$^+$ T8$^+$ cells (Cantor & Boyse, 1975) rather than of the Lyt 23$^+$ cells (the putative equivalent of OKT8$^+$ cells), also depressed in MG. In any case, the decrease of suppressor T cells is interesting with regard to the aetiopathogenesis of MG. One may conceive that it contributes to the overall hyperimmunity state characterized by the presence of multiple autoantibodies.

Our results also show that the presumably immature OKT4$^+$ T8$^+$ cells are present in excessive number in the blood of MG patients. These cells are not, however, very immature since most of them are OKT3$^+$, like peripheral T cells. They probably correspond to the last stage of intrathymic T cell differentiation. Their rapid disappearance in most patients after thymectomy, which fits with this hypothesis, also tells us that they are short-lived (perhaps because they transform into OKT3$^+$ T4$^+$ T8$^-$ and OKT3$^+$ T4$^-$ T8$^+$ cells), a data reminiscent of the disappearance of part of Lyt 123$^+$ cells after adult thymectomy in the mouse.

Finally, one should briefly discuss the relevance of our data to the therapeutic effects of thymectomy. The success of the operation in MG patients is a matter of diverse evaluation but is generally considered as significant (Papatestas *et al.*, 1971; Le Brigand *et al.*, 1972). Its

physiopathological basis is not well established; it may result from removal of a site of production of autoantibodies, especially anti-acetylcholine receptor antibodies, cessation of the production of a hormone depressing neuromuscular condition (thymopoietin), or removal of a site of intrathymic autosensitization of T cells against muscular structures (acetylcholine receptors present on epithelial cells or lymphocytes . . .). Our results indicate that thymectomy might also make a negative contribution to the evolution of the disease by depressing suppressor T cell function. It will be important to determine whether those patients with decreased suppressor T cells are less sensitive than those with normal suppressor T cells to the favourable effect of thymectomy.

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REFERENCES

ABDOU, N.I., LISAK, R.P., ZWEIMAN, B., ABRAHAMSOHN, I. & PENN, A.S. (1974) The thymus in myasthenia gravis. Evidence for altered cell populations. *N. Engl. J. Med.* **291,** 1271.

BACH, J.F. (1973) Evaluation of T cells and thymic serum factors in man using the rosette technique. *Transplant. Rev.* **16,** 196.

BIRNBAUM, G. & TSAIRIS, P. (1977) Thymic lymphocytes in myasthenia gravis. *Ann. Neurol.* **1,** 331.

CANTOR, H. & BOYSE, E.A. (1975) Functional subclasses of T lymphocytes bearing different Ly antigens. I. The generation of functionally distinct T cell subclasses is a differentiative process independent of antigen. *J. exp. Med.* **141,** 1376.

DRACHMAN, D.B., ANGUS, C.W., ADAMS, R.N. & KAO, I. (1978) Effect of myasthenia gravis. Immunoglobulin on acetylcholine receptor turnover: selectivity of degradation process. *Proc. Natl. Acad. Sci. USA,* **75,** 3422.

FELTKAMP, T.A.W., VAN DEN BERG-LOONEN, P.M., NIJENHUIS, L.E., ENGELFRIET, C.P., VAN ROSSUM, A.L., VAN LOGHEM, J.J. & OOSTERHUIS, H.J.G.H. (1974) Myasthenia gravis, autoantibodies, and HLA antigens. *Br. Med. J.* **i,** 137.

FRITZE, D., HERRMAN, C., NAEIM, F., SMITH, G.S. & WALFORD, R.L. (1974) HLA antigens in myasthenia gravis. *Lancet,* **i,** 240.

GALILI, U. & SCHLESINGER, M. (1974) The formation of stable E rosettes after neuraminidase treatment of either human peripheral blood lymphocytes or of sheep red blood cells. *J. Immunol.* **112,** 1628.

GOLDSTEIN, G. & MANGANARO, A. (1971) Thymin: a thymic polypeptide causing the neuromuscular block of myasthenia gravis. *Ann. N.Y. Acad. Sci.* **183,** 230.

HUANG, S.N., ROSE, J.W. & MAYER, R.P. (1977) Assessment of cellular and humoral immunity of myasthenics. *J. Neurol. Neurosurg. Psychiatry,* **40,** 1053.

ITOYAMA, Y., KAWANAMI, S., GOTO, I. & KUROIWA, Y. (1979) T and B lymphocytes in myasthenia gravis. *Clin. exp. Immunol.* **37,** 452.

KAO, I. & DRACHMAN, D.B. (1977) Myasthenia immunoglobulins accelerate acetylcholine receptor degradation. *Science,* **196,** 257.

KERBEL, R.S. & EIDINGER, D. (1972) Enhanced immune responsiveness to a thymus-independent antigen early after adult thymectomy: evidence for short-lived inhibitory thymus-derived cells. *Eur. J. Immunol.* **2,** 114.

KUNG, P.C., GOLDSTEIN, G., REINHERZ, E.L. & SCHLOSSMAN, S.F. (1979) Monoclonal antibodies defining distinctive human T cell surface antigens. *Science,* **206,** 347.

LE BRIGAND, H., LEVASSEUR, P., MERLIER, M., ROJAS-MIRANDA, A., GAUD, C. & NOVIANT, Y. (1972) Cent thymectomies chez des myasthéniques. Résultats lointains. *Rev. Neurol.* **126,** 267.

OPELZ, G., KEESEY, J., GLOUSKY, M. & GALE, R.P. (1978) Autoreactivity between lymphocytes and thymus cells in myasthenia gravis. *Arch. Neurol.* **35,** 413.

OSSERMAN, K.E. (1958) *Myasthenia Gravis.* Grune & Stratton, New York.

PAPATESTAS, A.D., ASPERT, L.I., OSSERMAN, K.E., OSSERMAN, R.S. & KARK, A.P. (1971) Studies in myasthenia gravis: effects of thymectomy. Results on 185 patients with non-thymomatous and thymomatous myasthenia gravis, 1941–1969. *Am. J. Med.* **50,** 465.

REINHERZ, E.H., KUNG, P.C., GOLDSTEIN, G. & SCHLOSSMAN, S.F. (1979a) A monoclonal antibody with selective reactivity with functionally mature thymocytes and all peripheral human T cells. *J. Immunol.* **123,** 1312.

REINHERZ, E.H., KUNG, P.C., GOLDSTEIN, G. & SCHLOSSMAN, S.F. (1979b) Separation of functional subsets of human T cells on a monoclonal antibody. *Proc. Natl. Acad. Sci. USA,* **76,** 4061.

REINHERZ, E.L., KUNG, P.C., GOLDSTEIN, G. & SCHLOSSMAN, S.F. (1980) A monoclonal antibody reactive with the human cytotoxic/suppressor T cell subset previously defined by a heteroantiserum termed TH_2. *J. Immunol.* **124,** 1301.

REINHERZ, E.H. & SCHLOSSMAN, S.F. (1980) The differentiation and function of human T lymphocytes. *Cell,* **19,** 821.

RICHMAN, D.P., PATRICK, J. & ARNASON, B.G. (1976) Cellular immunity in myasthenia gravis. Response to purified acetylcholine receptor and autologous thymocyte. *N. Engl. J. Med.* **294,** 694.

ROTTER, Y. & TRAININ, N. (1974) Thymus cell subpopulations exciting a regulatory function in the immune response of mice to polyvinylpyrrolidone. *Cell Immunol.* **13,** 76.

SAKANE, T. & GREEN, I. (1977) Human suppressor T

cells induced by Con A: suppressor T cells belong to distinctive T cell subclasses. *J. Immunol.* **119,** 1169.

STANLEY, E.F. & DRACHMAN, D.B. (1978) Effect of myasthenic immunoglobulin on acetylcholine receptors of intact neuromuscular functions. *Science*, **200,** 1285.

TERASAKI, P.J. & MILKEY, M.R. (1975) HLA haplotypes on 32 diseases. *Transplant. Rev.* **22,** 105.

WIJERMANS, P., OOSTERHUIS, H.J.G.H., ASTALDI, G.C.B., SCHELLEKENS, P.TH.A. & ASTALDI, A. (1980) Influence of adult thymectomy on immunocompetence in patients with myasthenia gravis. *J. Immunol.* **124,** 1977.

ZILKO, P.J., DAWKINS, R.L., HOLMES, K. & WITT, C. (1979) Genetic control of suppressor lymphocyte function in myasthenia gravis: relationship of impaired suppressor functions to HLA B8/DW3 and cold reactive lymphocytotoxic antibodies. *Clin. Immunol. Immunopathol.* **14,** 222.