Clin. exp. Immunol. (1978) 34, 111–117.

Immunoglobulins in myasthenia gravis. Kinetic properties of the acetylcholine-receptor antibody studied during lymph drainage

ANN KARI LEFVERT *Department of Clinical Chemistry, Karolinska sjukhuset, S-104 01 Stockholm, Sweden*

(*Received* 6 *February* 1978)

SUMMARY

A specific immunoglobulin, the receptor antibody, can be found in most patients with myasthenia gravis. In order to study the kinetic properties of this antibody, serial determinations of receptor antibody, total IgG and IgG 3 were made during drainage of thoracic duct lymph in three patients. The values obtained were used in a mathematical model to calculate some kinetic parameters. Values for T 1/2 and fractional rates of synthesis and catabolism obtained for total IgG and IgG 3 by this method were shown to agree with those found with other techniques. Most of the receptor antibody activity was found in the IgG 3 fraction but the receptor antibody had a shorter T 1/2 and higher fractional rates of synthesis and catabolism than IgG 3. These kinetic characteristics are consistent with rapid variations in plasma concentration of the receptor antibody. The cause of this rapid turnover could be strong antigenic stimuli and rapid elimination by the antigen, the cholinergic receptor protein.

INTRODUCTION

The first experimental evidence for the existence of a specific humoral factor in myasthenia gravis came in 1973 when it was shown that the myasthenic symptoms improved during thoracic duct lymph drainage and were aggravated by retransfusions of the cell free lymph (Bergström *et al.*, 1973). This humoral factor has now been identified as an antibody directed against cholinergic receptor structures on the motor end plate. The acetylcholine receptor antibody is present in 85–90% of patients with myasthenia gravis, but has not been found in other diseases (Appel, Almon & Levy, 1975; Lindström *et al.*, 1976; Monnier & Fulpius, 1977; Lefvert *et al.*, 1978). During lymph drainage and plasmapheresis (Pinching, Peters & Newsome Davis, 1976), as well as during immunosuppressive treatment with corticosteroids and azathioprine (Lefvert *et al.*, 1978), changes in clinical symptoms are well correlated to variations in receptor antibody concentration. The placental transfer of the receptor antibody from myasthenic mothers to their babies has been implicated in the disease neonatal myasthenia gravis (Keesey *et al.*, 1977). Recently, IgG and complement have been found bound to the postsynaptic region of the motor end plate in myasthenia gravis (Engel, Lambert & Howard, 1977).

A disease similar to human myasthenia gravis can be produced in animals by immunization with cholinergic receptor protein (Patrick & Lindstrom, 1973; Lennon, Lindstrom & Seybold, 1975). The human receptor antibody causes a disturbed neuromuscular function in mice (Toyka *et al.*, 1977) and aggravates the symptoms in rabbits with the experimental disease (Hammarström *et al.*, 1978).

All these data suggest that antibodies to the acetylcholine receptor are relevant to the pathogenesis of myasthenic symptoms. Knowledge of the metabolism of the receptor antibody should be of importance for the choice of appropriate therapy for patients with myasthenia gravis.

Correspondence: Dr Ann Kari Lefvert, Department of Clinical Chemistry, Karolinska sjukhuset, S-104 01 Stockholm, Sweden.

0099-9104/78/0100-0111$02.00

In this study, we have made serial determinations of receptor antibody and of normal immunoglobulins during thoracic duct lymph drainage in three patients. These values have been used in a mathematical model to calculate some kinetic parameters for the receptor antibody and for normal IgG.

MATERIALS AND METHODS

Preparation of partially purified cholinergic receptor from human skeletal muscle. Human skeletal muscle was collected from amputated legs at the time of operation. The muscle was immediately frozen and stored at −80°C. When used, the muscle was cut into small pieces and homogenized at ±0°C in 4 volumes of phosphate buffer, 0·05 mol/l with NaCl, 0·1 mol/l and EDTA 0·001 mol/l, pH 7·5 with the addition of Trasylol (Bayer AG, West Germany) to 10^6 Kallikrein inhibitory units/l. The homogenate was centrifuged at 30,000 *g* for 20 min at 4°C, the pellet washed once and resuspended in 3 volumes of the same buffer containing 1·5% Triton X-100. This mixture was stirred at room temperature for 90 min and then centrifuged at 30,000 *g* for 20 min at 4°C. The lipid layer was discarded and the supernatant collected and stored in aliquots at −80°C The ability to bind neurotoxin and acetylcholine-receptor antibody did not change during 4 months of storage.

The binding capacity for ^{125}I-α-neurotoxin (Naja naja siamensis) was determined by incubation of 1 ml of the supernatant with different amounts of toxin at 37°C for 1 hr, followed by gel filtration on Sephacryl G-200 superfine (Pharmacia Fine Chemicals, Sweden) to remove excess toxin. The recovery of toxin binding sites was 1–5 pmoles/g muscle (wet weight).

Iodination of neurotoxin. α-Neurotoxin from the venom of Naja naja siamensis was kindly supplied by Professor E. Karlsson, Uppsala University, Sweden. The neurotoxin was labelled with ^{125}I using the Chloramin-T method (Hunter & Greenwood, 1962). About 30 nmoles of neurotoxin (225 μg) was dissolved in 10 μl phosphate buffer (0·01 mol/l), pH 7·2, containing NaCl (0·14 mol/l), and mixed with 20–50 μl carrier-free Na ^{125}I (1·5 mCi). Chloramin-T solution (50 μl; 3·6 mmol/l in phosphate buffer, 0·05 mol/l, pH 7·5) was added and the solution thoroughly mixed. After 60 sec the reaction was terminated by the addition of sodium metabisulphite solution (100 μl; 53 mmol/l in phosphate buffer, 0·05 mol/l pH 7·5) and potassium iodine solution (200 μl; 60 mmol/l in phosphate buffer, 0·05 mol/l, pH 7·5). The iodinated neurotoxin was separated from excess reagents by gel filtration on Sephadex G-25 (Pharmacia Fine Chemicals, Uppsala, Sweden).

The specific radioactivity was calculated to be 13 Ci/mmol, assuming a quantitative recovery of protein during gel filtration. The labelled neurotoxin was stored in aliquots at −80°C.

Assay for receptor antibody. The amount of human skeletal muscle receptor preparation binding to IgG in the sample was measured by a method similar to that used by Appel, Almon & Levy, 1975, and by Lindstrom, 1977. The cholinergic receptor preparation, 10–20 pmoles, was incubated for 1 hr at 37°C with 200 pmoles of ^{125}I-α-neurotoxin. The labelled toxin-receptor complex was separated from excess toxin by gel filtration on Sephacryl S-200 superfine at room temperature. The column (2·5 × 40 cm) was equilibrated in phosphate buffer, 0·05 mol/l with NaCl, 0·1 mol/l and EDTA 0·001 mol/l, pH 7·4, with Triton X-100, 0·5% and Trasylol to 10^6 Kallikrein inhibitory units/l. Subsequently, 0·2–0·5 pmoles of the toxin-receptor complex was incubated with 5 μl of serum or plasma for 16 hr at 4°C. 300 μl of rabbit-anti-human IgG DACOPATTS A/S, Denmark) was added and the tubes were allowed to stand for 3 hr at 37°C. The precipitate was separated by centrifugation in a Beckman minifuge at 8000 *g* for 5 min, and was washed once with 1 ml of the same buffer. After alkaline hydrolysis and neutralization, the radioactivity and protein content of the precipitate was determined. Several dilutions of each sample and of the normal plasma pool were analysed to make sure that the assay was linear to the range used. The amount of receptor antibody (a) compared to normal IgG (b) in the sample was then calculated according to:

$$\frac{a}{b} = \frac{\text{CPM for patient sample} - \text{CPM for normal plasma}}{\text{CPM for normal plasma}}.$$

The CPM value for the normal plasma was corrected to the same amount of IgG as the patient sample. The concentration of receptor antibody (arbitrary units/l) was then calculated as: (total concentration of IgG) $\times \frac{a}{b}$: 10). This calculated value was related linearly to the amount of neurotoxin-receptor complex precipitated with myasthenic serum as calculated from the CPM values. Different preparations of muscle receptor varied in their binding capacity for both neurotoxin and for receptor antibody. This difference was minimized by using the calculated value for receptor antibody concentration in arb units/l. The normal plasma pool, which was the same in all experiments, was composed of plasma from fifty apparently healthy blood donors. Values above the upper 95% confidence limit of the normal pool were considered abnormal.

Protein determinations. These were performed according to Lowry *et al.*, 1951 using human serum albumin (Kabi AB, Stockholm, Sweden) as a standard.

Immunological IgG-determinations. These were made using an automated turbidimetric method. Rabbit antisera were obtained from DAKO-immunoglobulins, Denmark. Standard-human-serum, stabilized, from Behringwerke AG, West Germany, was used as a standard. The antiserum, 600 μl, diluted 1/30 in phosphate buffer, 0·05 mol/l, pH 7·4, with NaCl, 0·1 mol/l and polyethylene glycol, 90 g/l, was filtered (0·2 μm) and then mixed with the sample, 25 μl, diluted usually 1/168 in buffer without polyethylene glycol. The tubes were allowed to stand in room temperature for 45 min. Blanks were prepared for each sample. The turbidity was measured at 340 nm using a LKB 2074 calculating absorptiometer (LKB, Sweden). The normal range of IgG using this method was found to be 7–15 g/l.

Preparation of IgG fraction from lymph or plasma. This was performed as described previously (Lefvert & Bergström, 1977).

Separation of IgG subclasses. This was performed by affinity chromatography on Protein-A-Sepharose (Pharmacia Fine Chemicals,Sweden) as described by Hjelm (1975).

Collection of samples during lymph drainage. Lymph was collected with heparin as an anticoagulant at 4°C. The lymph was centrifuged at 2000 *g* to remove cells within 24 hr and stored at −80°C. Plasma samples with EDTA as an anticoagulant as well as serum samples were stored at −80°C until analysed.

Patients. Three patients, two females, SK and JJ, 18 and 28 years old, and one male, BA, 62 years old, were thymectomized. In the same session, drainage of thoracic duct lymph was started. Duration of severe generalized myasthenic symptoms were 6 months, 4 years and 9 months, respectively, before the operation. No medication other than cholinesterase inhibitors had been given to the patients. Plasma volumes were calculated from body weights.

Mathematical model. The model is based on the following assumptions: (i) rapid equilibrium between intra- and extravascular pools; (ii) concentration-dependent catabolism of IgG (Waldmann & Strober, 1969) and (iii) constant synthesis of IgG during lymph drainage (Strober *et al.*, 1967).

We denote by x the concentration of the component studied (g/l for IgG 3 or arb. units/l for receptor antibody) in a plasma pool of constant volume V (1). If the rate of synthesis is α (g/l/day) and the relative catabolic rate is γ x (/day) at the concentration x, then the small change V × dx, in the amount of the component is, during a short time dt:

$$V \times dx = \alpha \times V \times dt - \gamma x \times dt \times Vx - \theta \times dt \times kx \tag{1}$$

where we have also taken into account a lymph drainage at constant rate θ (1/day) and estimated the concentration gradient between plasma and lymph with the constant factor $k<1$. (This factor is found experimentally).

Equation (1) leads to the non linear differential equation:

$$\frac{dx}{dt} = \alpha - \gamma x^2 - kfx \tag{2}$$

where $f = \theta V$ (/day). The solution of equation (2) is, in the general case,

$$x(t) - x_p = \frac{2\beta}{\gamma} \times \frac{e^{-2\beta t}}{A - e^{-2\beta t}} \tag{3}$$

where x_p is the final plateau value and

$$\beta = \sqrt{\alpha\gamma + 0{\cdot}25 f^2 k^2}$$
$$A = \frac{1 + x_p/x_o}{1 - x_p/x_o} + \frac{2\beta}{\gamma x_o} \times \frac{1}{1 - x_p/x_o}$$
$$x_o = x(o)$$

In our experiment we verified that $A >> 1$ meaning that equation (3) is, in good approximation,

$$x(t) - x_p = \frac{2\beta}{A\gamma} \times e^{-2\beta t}$$

Plotting log $[x(t) - x_p]$ against t we may therefore calculate the slope, $s = 2\beta$, from the experiment. Now, for large times when the plateau value is almost reached, the time variation $\frac{dx}{dt} \sim 0$ and we have from equation (2):

$$0 = \alpha - \gamma x_p^2 - f k x_p$$

This equation combined with that for s gives us

$$\alpha = 0{\cdot}5 x_p (s + kf) \tag{4}$$

which in a simple way determines the rate of synthesis from the experimental data.

Another kinetic parameter of interest is the half life of the component. Consider the experimental situation where a radiolabelled component is injected into the concentration x_i (g/l) and its subsequent catabolism is followed. The concentration (g/l) of the radioactive component in the plasma in our model is governed by $\frac{dx}{dt} = -\gamma(x + x_o)x$ where x_o is, as before, the equilibrium plasma concentration before injection. The solution is $x(t) = x_i \times \frac{e^{-\gamma x_o t}}{1 + \frac{x_i}{x_o}(1 - e^{-\gamma x_o t})}$.

If the injected amount is small compared to the equilibrium amount, that is, if $x_i/x_o << 1$ then we have $x(t) \sim x_i e^{-\gamma x_o t}$ and the catabolic rate may be adequately described with a half life $T\,1/2 = \frac{\ln 2}{\gamma x_o}$ (days).

Returning to equation (2), without drainage ($f = 0$) we see that for large times, when $\frac{dx}{dt} \sim 0$ the equilibrium plasma value is $x_o = \sqrt{\alpha/\gamma}$.

Therefore the half-life is also:

$$T\,1/2 = \frac{\ln 2}{\alpha} \times x_o \text{ (days)} \tag{5}$$

To calculate the fractional catabolic rate (FCR) of IgG 3 and receptor antibody in the steady state condition (without drainage), (Matthews, 1957) we relate, for each component, the total amount synthesized per day to the amount in the plasma pool:

$$FCR = \frac{\alpha \times V_{tot}}{x_o \times V} \tag{6}$$

where V_{tot} is the total body protein pool and V the intravascular protein pool (c.f. equation (1)). Since IgG 1, 2, 4 have similar kinetic properties (Morell, Terry & Waldmann, 1970) the concentration of the total IgG can be written as $x = x_1 + x_2$ (g/l) where x_1 refers to IgG 3 and x_2 to the rest. For each component x_1 and x_2 we have an equation like equation (2) with different values for α, γ and k. It is not possible to give a simple solution of the sum x within this model.

However, we may still extract some information from the experiments. In equilibrium we have $x_o = x_{1_o} + x_{2_o}$. The total rate of synthesis is $\alpha = \alpha_1 + \gamma_2 \times {x_{2_o}}^2$ from which follows $\gamma_2 = \frac{\alpha - \alpha_1}{(x_o - x_{1_o})^2}$. As IgG3 is only a small ($\sim$ 5%) fraction of the total IgG, the T 1/2 of total IgG will be approximately equal to T 1/2 for x_2, that is :

$$T\,1/2 \text{ (total IgG)} \sim \frac{\ln 2}{\gamma_2 \times x_{2o}} \tag{7}$$

Summary. The kinetic parameters α (rate of synthesis) and T 1/2 for IgG 3 and receptor antibody are calculated from equations (4) and (5) using the value before the drainage (x_0) ,the value at the plateau after 4–5 days of drainage (x_p) and the slope of the curve (s) (see Figs 1, 2 and 3).

Now, if we want the total rate of synthesis (mg or arb. units/kg body weight/day) and the fractional catabolic rate (fraction of the intravascular pool catabolized per day, c.f. equation (6)), it is necessary to know the distribution of the immunoglobulin between the intra- and extravascular pools. As this cannot be obtained from our experimental data we have used the same value, taken from Morell, Terry & Waldmann, 1970, for IgG 3 and for the receptor antibody, since the receptor antibody activity was found in an IgG 3 fraction.

The amount of total IgG does not follow a model as simple as that given in equation (2). From our experiment we can determine only one of the two kinetic parameters α and T 1/2. We have chosen to take a value for α from the work of Solomon, Waldmann & Fakey, 1963, and Wochner *et al.* 1966 and calculate the approximative T1/2 from this and from our experimental results according to equation (7).

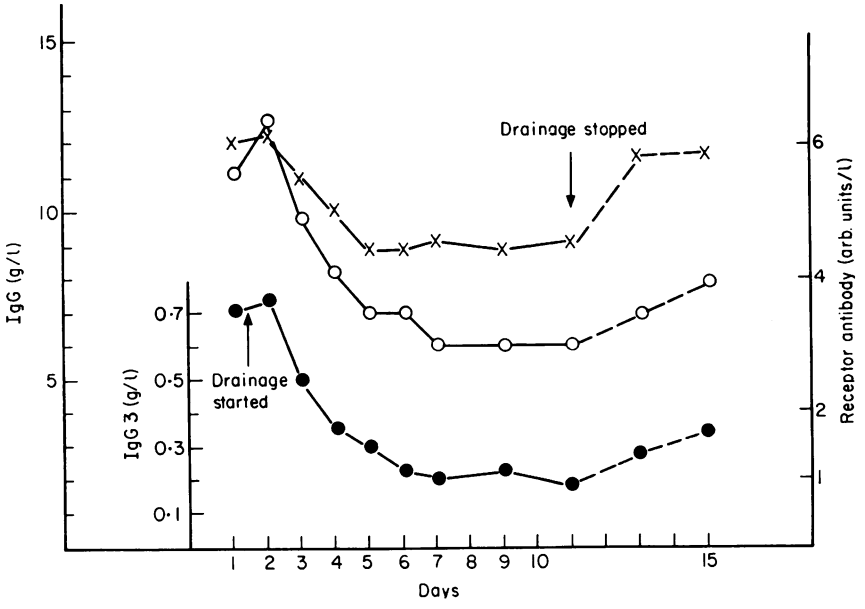

FIG. 1. Concentrations of total IgG (○), IgG 3 (●) and receptor antibody (X) in patient S.K. during and after lymph drainage (1·0 l/day). Body weight: 47kg. Plasma volume: 2·10 l.

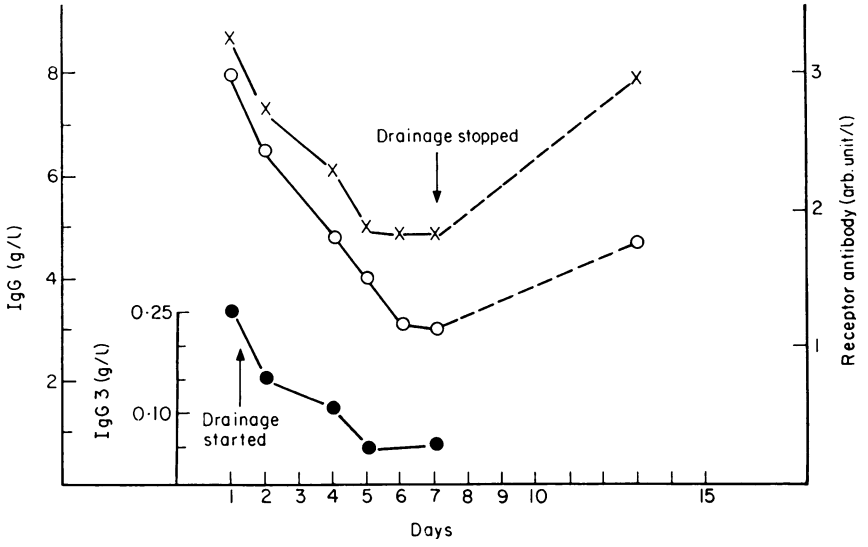

FIG. 2. Concentrations of total IgG (○), IgG 3 (●) and receptor antibody (X) in patient J.J. during and after lymph drainage (1·7 l/day). Body weight: 50 kg. Plasma volume: 2·25 l.

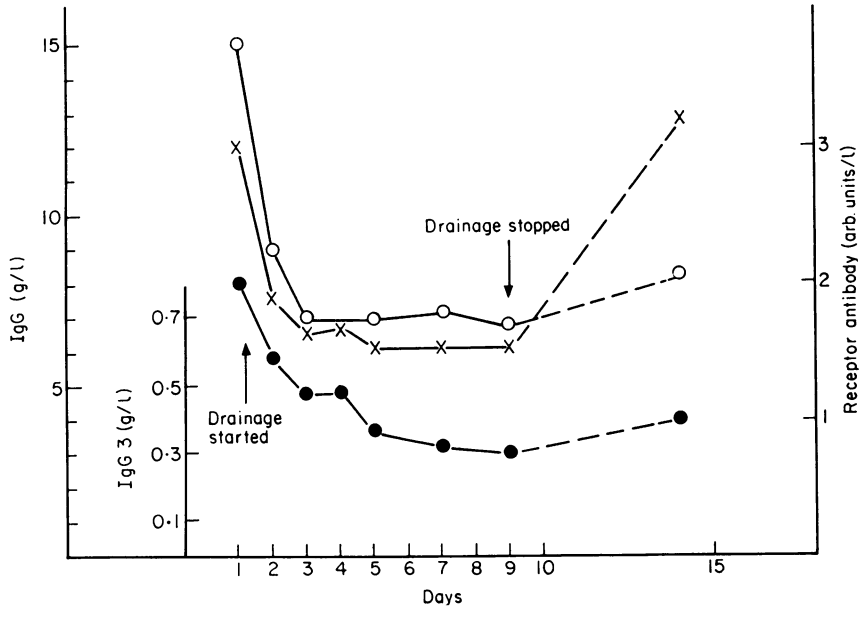

FIG. 3. Concentrations of total IgG (○), IgG 3 (●) and receptor antibody (X) in patient B.A. during and after lymph drainage (1·2 l/day). Body weight: 81 kg. Plasma volume: 3·80 l.

RESULTS

The amount of lymph drained for patient SK was 1·0 l/day, for patient JJ, 1·7 l/day and for patient BA, 1·2 l/day. These volumes were constant during the drainage period. The concentration gradient, k, (see equation (1)) was determined from the plasma and lymph values for the respective IgG component, and was found to be 0·7–0·8 in all cases.

A decreased concentration of IgG, IgG 3 and receptor antibody in plasma was evident within 24–36

TABLE 1. Kinetic parameters of receptor antibody, IgG 3 and total IgG in three patients with myasthenia gravis

	Patient S.K.	Patient J.J.	Patient B.A.	Values from the work of Morell, Terry & Waldmann (1970)
T 1/2 (days) receptor antibody	2·0	2·5	1·5	
T 1/2 (days) IgG 3	5·0	6·5	4·5	7·0±1
T 1/2 (days) total IgG	24·0	15·0	37·0	21±5
Rate of synthesis (arb. units/kg/day) receptor antibody	0·140	0·062	0·101	
Rate of synthesis (mg/kg/day) IgG 3	6·9	1·9	8·0	3·4±0·7
Fractional catabolic rate (%) receptor antibody	52·0	43·0	72·0	
Fractional catabolic rate (%) IgG 3	21·0	17·0	23·0	17·0

hr after the operation (Fig 1, 2 and 3). Plateau values were established for all three curves within 4–5 days. The percentage decrease was greatest for IgG 3, 60–80%, and least for the receptor antibody 30–50%. In all patients, most of the receptor antibody activity was found in IgG 3 (Lefvert & Bergström, 1977).

All patients improved markedly during the drainage period, but returned to their pre-operative state within a few days after the drainage was stopped at 10, 8 and 6 days, respectively. At the same time, the amount of receptor antibody in the plasma had reached pre-operative values. The concomitant increase of total IgG and IgG 3 was much slower.

The T 1/2 and rates of synthesis and fractional catabolism for the receptor antibody and IgG 3 were calculated from experimental data only (Table 1) (c.f. equations (4), (5) and (6)) using the value of 64% for the intravascular distribution (Morell, Terry & Waldmann, 1970). To calculate the T 1/2 of the total IgG, the rate of synthesis of 34 mg/kg/day was used (Solomon, Waldmann & Fakey, 1963; Wochner *et al.*, 1966) (c.f. equation (7)).

The receptor antibody was shown to have a more rapid turnover, with short T 1/2 and rapid rates of synthesis and catabolism, than the total IgG and IgG 3 in all three patients.

DISCUSSION

There is a good correlation between the kinetic parameters for IgG3 and normal IgG when calculated according to the model and results found with other techniques (Waldmann & Strober, 1969).

For the calculation of T 1/2 for total IgG, we have used the value 34 mg/kg/day for the rate of synthesis (Solomon, Waldmann & Fakey, 1963; Wochner *et al.*, 1966). The rate of synthesis of IgG is regulated mainly by antigenic stimuli (Cohen, McGregor & Carrington, 1961), which can be regarded as constant in these patients. The rate of synthesis does not depend on the concentration of IgG. Even large losses of IgG as, for example, in patients with intestinal lymphangiectasia, do not lead to an increased rate of synthesis (Strober *et al.*, 1967). Therefore, the assumption in the model that the rate of synthesis of IgG is not changed during the lymph drainage would seem to be reasonable.

The fractional rate of synthesis and the fractional catabolic rate of the receptor antibody is about two to four times that of IgG 3. If we assume, that less than 64% of the receptor antibody is located in the plasma pool, the figures would be even higher. Even if the receptor antibody was located exclusively in the plasma pool, the calculated fractional rates of synthesis and catabolism would still be larger than those for IgG 3.

In all patients the receptor antibody was found to have similar kinetic characteristics: a short T 1/2, a rapid rate of synthesis and a high fractional catabolic rate. These properties are consistent with rapid variations in the concentration of receptor antibody in plasma, which has been reported during treatment with corticosteroids and ACTH (Lefvert & Matell, 1977).

The fast turnover rate of the receptor antibody found in our patients might also have important therapeutic implications. Procedures which increases the catabolism of IgG such as lymph drainage and plasmapheresis, reduce the concentration of normal IgG more effectively than the concentration of a

receptor antibody with a much higher fractional rate of synthesis. On the other hand, treatment with corticosteroids which leads to a decreased rate of synthesis of immunoglobulins molecules (Germuth, Oyama & Ottinger, 1951), lower the concentration of receptor antibody relatively more than the concentration of normal IgG (Lefvert *et al.*, 1978), and thus might be a more rational therapy for this disease.

It is tempting to speculate that the more rapid turnover of the receptor antibody than of other IgG-fractions is caused by strong antigenic stimuli and a rapid elimination from circulation by the antigen, the cholinergic receptor structures. This would support the theory, that the receptor antibody is involved in the pathogenesis of the myasthenic symptoms.

The author wishes to thank Dr T. Lefvert for valuable advice regarding the mathematical model and Drs K. Bergstrom and G. Matell for critically reviewing this manuscript.

Part of this work was presented as a poster at the Neutrochemical Group Workshop on Neuromuscular Disorders, the University of Bath, 30 March to 1 April 1977.

REFERENCES

APPEL, S.H., ALMON, R.R. & LEVY, N. (1975) Acetylcholine receptor antibodies in myasthenia gravis. *N. Engl. J. Med.* **293**, 760.

BERGSTRÖM, K., FRANKSON, C., MATELL, G. & VON REIS, G. (1973) The effect of thoracic duct lymph drainage in myasthenia gravis. *Europ. Neurol.* **9**, 157.

COHEN, S., MCGREGOR, I.A. & CARRINGTON, S. (1961) Gamma-globulin and aquired immunity to human malaria. *Nature (Lond.)* **192**, 733.

ENGEL, A.G., LAMBERT, E.H. & HOWARD, F.M. (1977) Immune complexes (IgG and C3) at the motor end-plate in myasthenia gravis. Ultrastructural and light microscopic localization and electrophysiologic correlations. *Mayo Clinic Proceedings*, **52**, 267.

GERMUTH, F.G., OYAMA, J. & OTTINGER, B. (1951) The mechanism of action of 17-hydroxy-11-dehydrocorticosterone (compound E) and of the adrenocorticotropic hormone in experimental hyper-sensitivity in rabbits. *J. exp. Med.* **94**, 139.

HAMMARSTRÖM, L., HEILBRONN, E., HILTON-BROWN, P., LEIVERT, A.K., MATELL, G., MATTSON, C., SMITH, E., STÅHLBERG, E. & UDDGÅRD, A. (1978) Pathological mechanisms in myasthenia gravis: transfer of myasthenic symptoms to rabbits with human IgG. Submitted for publication.

HJELM, H. (1975) Isolation of IgG 3 from normal human sera and from a patient with multiple myelomatosis. *Scand. J. Immunol.* **4**, 633.

HUNTER, W.M. & GREENWOOD, F.C. (1962) Preparation of iodine ^{131} labelled human growth hormone of high specific gravity. *Nature (Lond.)* **194**, 495.

KEESEY, J., LINDSTROM, J., COKELY, H. & HERRMANN, C. (1977) Anti-acetylcholine receptor antibody in neonatal myasthenia gravis. *N. Engl. J. Med.* **296**, 55.

LEFVERT, A.K. & BERGSTRÖM, K. (1977) Immunoglobulins in myasthenia gravis. Effect of human lymph IgG 3 and $F(ab')_2$ fragments on a cholinergic receptor preparation from Torpedo marmorata. *Eur. J. Clin. Invest.* **7**, 115.

LEFVERT, AK., BERGSTRÖM, K., MATELI, G., OSTERMAN, P.O. & PIRSKANEN, R. (1978) Determination of acetylcholine receptor antibody in myasthenia gravis: Clinical usefulness and pathogenetic implications. *J. Neurol. Neurosurg. Psychiat.* **41**, 394.

LEFVERT, AK. & MATELL, G. (1977) Antibodies to human cholinergic receptor proteins in patients with myasthenia gravis: studies during immunosuppressive treatment. *Acta med. Scand.* **201**, 181.

LENNON, V.A., LINDSTROM, J.M. & SEYBOLD, M.E. (1975) Experimental autoimmune myasthenia: a model of myasthenia gravis in rats and guinea pigs. *J. exp. Med.* **141**, 1365.

LINDSTROM, J.M., SEYBOLD, M.E., LENNON, V.A., WHITTINGHAM, S. & DUANE, D.D. (1976) Antibody to acetylcholine receptor in myasthenia gravis. Prevalence, clinical correlates and diagnostic value. *Neurology*, **26**, 1054.

LINDSTROM, J. (1977) An assay for antibodies to human acetylcholine receptor in serum from patients with myasthenia gravis. *Clin. Immunol. Immunopath.* **7**, 36.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265.

MATTHEWS, C.M.E. (1957) The theory of tracer experiments with ^{131}I-labelled plasma proteins. *Phys. med. Biol.* **2**, 36.

MONNIER, V.M. & FULPIUS, B.W. (1977) A radioimmunoassay for the quantitative evaluation of anti-human acetylcholine receptor antibody in myasthenia gravis. *Clin. exp. Immunol.* **29**, 16.

MORELL, A., TERRY, W.D. & WALDMANN, T.A. (1970) Metabolic properties of IgG subclasses in man. *J. Clin. Invest.* **49**, 673.

PATRICK, J. & LINDSTROM, J. (1973) Autoimmune response to acetylcholine receptor. *Science*, **180**, 871.

PINCHING, A.J., PETERS, D.K. & NEWSOM DAVIS, J. (1976) Remission of myasthenia gravis following plasma exchange. *Lancet*, **ii**, 1373.

SOLOMON, A., WALDMANN, T.A. & FAKEY, J. (1963) Metabolism of normal 6·6 S gamma-globulin in normal subjects and in patients with macroglobulinemia and multiple myeloma. *J. Lab. clin. Med.* **62**, 1.

STROBER, W., WOCHNER, R.D., CARBONE, P.P. & WALDMANN, T.A. (1967) Intestinal lymphangiectasia. A protein-losing enteropathy with hypogammaglobulinemia, lymphocytopenia and impaired homograft rejection. *J. Clin. Invest.* **46**, 1643.

TOYKA, K.V., DRACHMAN, D.B., PESTRONK, A. & KAO, I. (1975) Myasthenia gravis: passive transfer from man to mouse. *Science*, **190**, 397.

WALDMANN, T.A. & STROBER, W. (1969) Metabolism of immunoglobulins. *Progr. Allergy*, **13**, 1.

WOCHNER, R.D., DREWS, O., STROBER, W. & WALDMANN, T.A. (1966) Accelerated breakdown of immunoglobulin G (IgG) in myotonic dystrophy. A hereditary error of immunoglobulin catabolism. *J. Clin. Invest.* **45**, 321.