PATHOLOGICAL MECHANISMS IN EXPERIMENTAL AUTOIMMUNE MYASTHENIA GRAVIS

II. Passive Transfer of Experimental Autoimmune Myasthenia Gravis in Rats With Anti-Acetylcholine Receptor Antibodies*

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Immunization of mammals with purified acetylcholine receptor protein (AChR)[1] results in an autoimmune response to skeletal muscle AChR which impairs neuromuscular transmission, causing experimental autoimmune myasthenia gravis (EAMG). In the introduction to the accompanying article (1) the striking similarities between EAMG and the human disease myasthenia gravis (MG) were reviewed. In that article we showed that immunization of rats with purified syngeneic AChR protein or AChR from fish electric organs induced an autoimmune response to muscle AChR. Antibodies to AChR were found both free in serum and bound to muscle AChR. Also, a large decrease in AChR content was noted. In this paper we investigate the role of antibodies to AChR in the impairment of neuromuscular transmission in EAMG by passively transferring antibody from rats with EAMG to normal rats.

Passive transfer experiments are intended to clarify the relative contribution of cell-mediated and humoral immunity to causing the changes in AChR function, AChR content, and postsynaptic membrane structure which are responsible for impairment of neuromuscular transmission. Previous experiments suggest that passive transfer of EAMG with antibody should be possible. Antisera from animals with EAMG block AChR activity on muscle (2, 3) and electric organ cells (4–6) in vitro. Chronic administration to mice of large amounts of immunoglobulin from patients with MG resulted in decreased miniature end-plate potential amplitude and diminished toxin binding to end-plates (7). High titers of antibody to AChR have been found in the serum of babies suffering from

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[1] *Abbreviations used in this paper*: AChR, acetylcholine receptor protein; EAMG, experimental autoimmune myasthenia gravis; mepps, minature end-plate potentials; MG, myasthenia gravis.

neonatal myasthenia (6). Although transplacental transfer of this antibody from the mother to the baby may be responsible for the transient myasthenia observed in the baby, it is also possible that sensitized lymphoid cells from the mother invaded the baby. EAMG can be passively transferred with sensitized lymphocytes (8, 9), but this remains inconclusive since antibody-producing cells might be transferred as well.

In this paper we show that EAMG can be passively transferred between rats with antibody to AChR. The disturbances at the neuromuscular junction in rats receiving antibody are very similar to those observed in rats suffering from acute EAMG. Acute EAMG occurs 8–10 days after immunization of rats with AChR (10) and is characterized by massive invasion of the end-plate by phagocytic cells (11, 12). The appearance of phagocytic cells at the end-plates of rats receiving antibody to AChR shows that the cell-mediated response at the end-plate during acute EAMG is antibody dependent. Phagocytic cells are not observed during the transient remission from acute EAMG, nor are they observed during chronic EAMG (9, 10) which usually begins coincident with a large increase in humoral antibody to muscle AChR around day 28 (6).

Studies of rats with passively transferred EAMG reveals the existence of two control mechanisms acting in animals surviving with chronic EAMG. One mechanism terminates the phagocytic invasion of the end-plate despite continued presence of antibody. Another mechanism appears to alter turnover of AChR to insure a critical number of functional AChR despite the massive excesses of antibody to AChR present.

Materials and Methods

Inoculation of Donor Rats. Female Lewis rats aged 10 wk were immunized with 15 μg of AChR from *Electrophorus electricus* (eel) in complete Freund's adjuvant and with *Bordetella pertussis* vaccine as additional adjuvant as previously described (10). Control rats received adjuvants only.

Preparation of Antibodies. Sera obtained 25 or more days after inoculation were stored at −20°C. Crude immunoglobulin fractions were prepared from pooled sera by precipitation with 40% $(NH_4)_2SO_4$; IgG was prepared by chromatography on DEAE cellulose. Crude immunoglobulin was used in all experiments unless otherwise specified. Dilutions were made from a stock solution 3.4-fold concentrated with respect to serum. The stock contained 59.4 mg/ml of protein and antibody to eel AChR at a concentration of 2.42×10^{-6} M, of which 1.86×10^{-7} M also recognized AChR from rat muscle. For control experiments immunoglobulin from rats immunized only with adjuvant was diluted from a 3.2-fold concentrated stock containing 47.9 mg/ml of protein.

Anti-AChR Antibody Assay. Antibodies to rat muscle AChR were assayed by a previously described indirect coprecipitation radioimmunoassay employing as antigen junctional muscle AChR solubilized from normal rats and complexed with ^{125}I-α-bungarotoxin (6). Antibody concentrations were expressed in moles of ^{125}I-α-bungarotoxin-binding sites precipitated per liter of serum. The total amount of antibody contained in the serum of individual rats was calculated assuming a serum vol of 5 ml.

Complement Fixation Assay. For sensitizing indicator sheep red blood cells (SRBC), a dilution of heated rabbit anti-SRBC antiserum was selected which would completely lyse, but not agglutinate, SRBC in the presence of sufficient complement. Fresh guinea pig serum, absorbed with SRBC to remove "natural antibody", was used as a source of complement at a concentration causing less than 100% lysis of sensitized SRBC. Rat anti-eel AChR serum (obtained in the chronic phase of EAMG, with a titer of 1.2×10^{-5} M) and adjuvant control rat serum were heated at 56°C for 30 min before testing. Eel AChR in Triton X-100 (0.5%) was serially diluted 10-fold in phosphate-buffered saline, and Triton X-100 (0.5%) was serially diluted to provide a control for any hemolytic or anti-complementary activity. Sera, AChR solutions, and diluted guinea pig comple-

ment were mixed at ratios 1:1:2. After incubation at 37°C for 45 min, an aliquot of each was tested for residual complement activity by mixing with sensitized SRBC. The end points of hemolysis were read visually after being left at 37°C for 30 min, then overnight at 4°C.

Injection of Antibody. Antibody was injected in a vol of 1 ml in normal saline into the jugular vein of female Lewis rats anesthetized with ether.

Clinical Evaluations. After injection of antibody, rats were examined every 12 h for signs of weakness and fatigability which were scored on a scale ranging from + from weak cry and grip to +++ for moribund (10). Rats were weighed daily.

Electromyographic Evaluations. The amplitude of the compound muscle action potential evoked by a supramaximal nerve stimulus was measured in both forelimbs, as described previously (13). A decrement in amplitude of the response during repetitive stimulation at 5/s was expressed as the ratio of the fifth to the first response.

Microelectrode Studies. Intracellular microelectrodes were used to record miniature end-plate potentials and end-plate potentials evoked by nerve stimulus in muscle fibers of forelimb toe flexors and diaphragm as described previously (14). In each muscle the mean amplitude of 25 miniature end-plate potentials was determined in each of an average of 20 fibers.

Histological Studies. Formalin post-fixed cryostat sections of forelimb muscles were reacted for cholinesterase with α-napthyl acetate as substrate and lightly counterstained with hematoxylin, as previously described (11, 12).

Quantitation of Total Muscle AChR. AChR was extracted from rat carcasses with Triton X-100, labeled with ^{125}I-α-bungarotoxin, and precipitated by sequential addition of excess rat antibodies to muscle AChR and goat anti-rat immunoglobulin as described in the accompanying paper (1).

Quantitation of Muscle AChR Complexed In Situ with Antibody. Concentration of antibody-AChR complexes in muscle extracts labeled with ^{125}I-α-bungarotoxin was determined by direct precipitation with goat anti-rat immunoglobulin as described in the accompanying paper (1).

Results

Clinical Observations. Within 12 h after receiving a single intravenous injection of 10.7×10^{-11} mol of anti-rat muscle AChR antibody, six of six recipient rats examined in detail showed clinical signs of weakness and fatigability, and the hunched posture and cupped hands characteristic of EAMG (10). Clinical signs were maximal at 48–60 h and began to diminish by 72 h, but recovery was not complete until later than 7 days after injection of antibody (Fig. 1). Within 24 h of antibody transfer, characteristic signs of chronic respiratory disease (porphyrin staining of tears and nasal secretions) became overt, peaking at 48 h and beginning to resolve at 72 h. Loss of weight, which did not occur in rats receiving immunoglobulin from donors immunized only with adjuvants (Fig. 2), had already begun at 24 h and peaked at 72 h. The weight loss probably reflected tissue dehydration due to the rats' inability to raise (or sustain raised) their heads to the water bottles, but some degree of calorie deficiency may have occurred if chewing and swallowing were also impaired. Weight was not completely regained until more than a week later. The severity of clinical signs increased with increasing doses of antibody (Fig. 5). IgG purified from the crude immunoglobulin fraction used for these studies was equally efficient in its capacity to transfer EAMG (Table I).

Electrophysiological Signs of Passive Transfer. Evidence of a defect of neuromuscular remission in rats injected with antibody to AChR was obtained by electromyography (Fig. 3). As in acute EAMG (14), but not chronic EAMG, the amplitude of compound muscle action potential evoked in forearm muscles by a supramaximal nerve stimulus was reduced to as low as 5% of normal on the 2nd

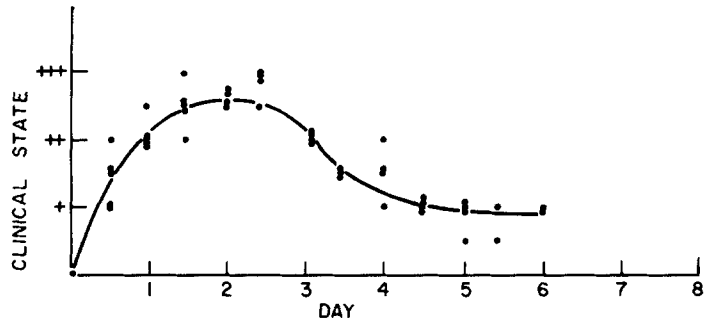

FIG. 1. Time-course of clinical signs of EAMG after a single injection of antibody to AChR. Rats received a dose of 10.7×10^{-11} mol of antibody to rat AChR i.v. at time zero and were tested for clinical signs of EAMG at 12-h intervals thereafter.

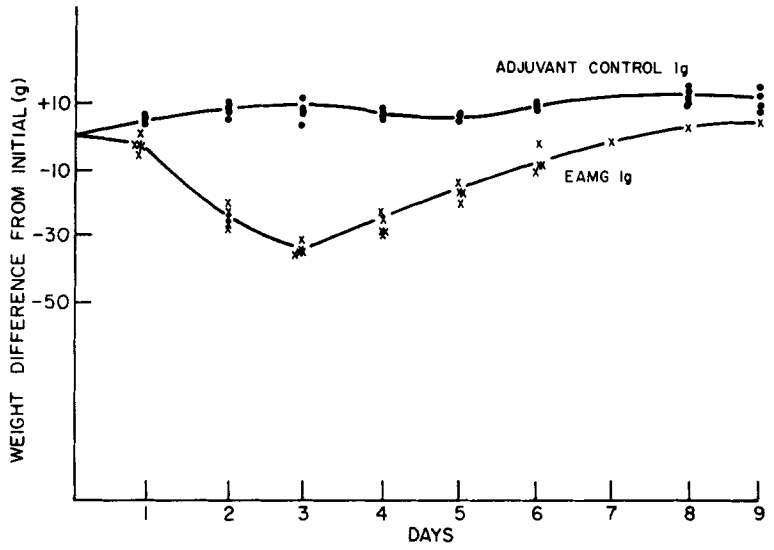

FIG. 2. Weight loss in rats receiving antibody to AChR. Rats in both groups received immunoglobulin i.v. at time zero. One group (EAMG Ig) received 10.7×10^{-11} mol of antibody to rat AChR. The other group (adjuvant control Ig) received an equal number of milligrams of protein (27 mg) from serum of rats immunized only with adjuvants.

day (Fig. 3). This suggested that transmission had failed completely at many nerve-muscle junctions, leaving many muscle fibers functionally denervated. Also, as in acute EAMG (13), a decrement of the muscle response occurred during repetitive nerve stimulation (Fig. 3). This indicated that transmission failed in an increasing proportion of muscle fibers after the first response. The fifth response fell to an average of 70% of the first response, which is as large a decrement as observed in acute EAMG. A decrement in excess of 10% is considered significant (13). The time-course of severity was approximately parallel for clinical signs (Fig. 1), muscle action potential amplitude, and electromyographic decrement.

Transmission was studied at individual neuromuscular junctions using microelectrodes in both the forelimb muscles, which had been studied by electro-

TABLE I

*Comparison of Passive Transfer of EAMG by Crude Immunoglobulin and Purified IgG**

	Crude immunoglobulin	Purified IgG
Dose	7.0×10^{-11} mol	7.3×10^{-11} mol
Clinical state day 2	+	++
Amount of AChR	4.52×10^{-11} mol/rat	2.23×10^{-11} mol/rat
Amount antibody-AChR	0.802×10^{-11} mol/rat	0.478×10^{-11} mol/rat
Amount serum antibody	1.35×10^{-11} mol/rat	1.57×10^{-11} mol/rat

* Two rats were given each dose and then evaluated on day 2.

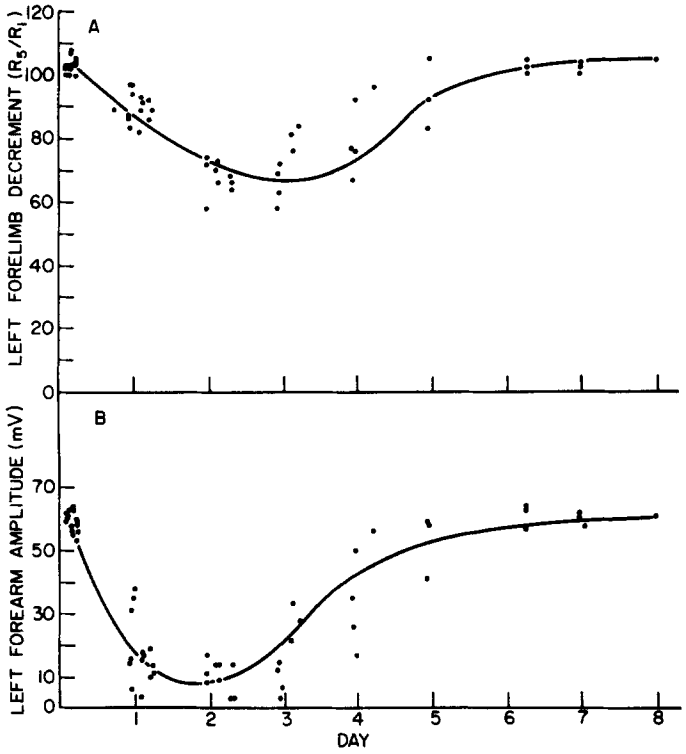

FIG. 3. Time-course of electromyographic signs after a single injection of antibody to AChR. (A) shows the decrement in electromyogram amplitude of the left forelimb of rats at intervals after receiving 10.7×10^{-11} mol of antibody to rat AChR at time zero. (B) shows the average amplitude of the first response of the left forelimb of the same rats. These are the same animals used in Fig. 6.

myography, and also in the diaphragm. As in acute EAMG (14), the forelimb muscles were more severely affected than diaphragm. The pattern of abnormalities in a rat 2 days after receiving anti-AChR antibody was like that seen in the acute phase of actively induced EAMG. When the diaphragm was transilluminated, the end-plate zone had a greyish appearance which occurred in acute EAMG and was related to invasion of this zone by phagocytic cells (11, 12) and focal degeneration of the muscle fibers. Miniature end-plate potentials (mepps) produced by spontaneous release of ACh quanta from the nerve ending were not observed in many fibers, especially in forearm muscles. Failure to observe

mepps suggested that nerve contact with muscle might be interrupted by phagocytic cells, as in the case of acute EAMG (11, 12). Consistent with this interpretation was the observation that in many fibers nerve stimulation produced no end-plate potential or action potential, although the fibers responded to direct stimulation. The average number of quanta released from the nerve endings by a nerve impulse in fibers with end-plate potentials was only 48 (normal 159–203 [14]) in the forearm muscle of a rat 2 days after injection. Such low values were seen only in acute phase of EAMG (14) and are consistent with the interpretation that only small areas of presynaptic membrane remain in contact with persisting residues of the postsynaptic membrane which escaped destruction during cellular invasion. End-plate potentials were severely reduced in amplitude in forelimb muscles, and therefore frequently did not trigger a muscle fiber action potential. Although end-plate potentials in the diaphragm 48 h after antibody transfer were sufficient to trigger action potentials, neuromuscular transmission was blocked by only one-fifth the amount of curare normally required. As in both acute and chronic EAMG, the amplitude of those mepps which could be recorded was reduced. Fig. 4 shows that the time-course for this effect was similar to that for clinical and electromyographic effects.

Microscopic Studies. Direct evidence of invasion of the end-plate zone by phagocytic cells, as in acute EAMG, was obtained by microscopy (Fig. 5). Postsynaptic membrane regions were found split away from the muscle fiber, and macrophages were observed interposed between nerve ending and muscle fiber. As in acute EAMG (11, 12) some of the fibers showed segmental necrosis and invasion by macrophages. This began immediately adjacent to the end-plate and extended from there for distances of up to 200 μm in either direction. Splitting of the postsynaptic region from the muscle fiber and the cellular reaction were first observed at 24 h. At this time only a few fibers showed segmental necrosis. By 48 h more than half of the end-plates were splitting from the fibers or were invaded by macrophages, and about 10% of the fibers showed segmental necrosis. Between 48 h and 5 days the cellular reaction almost completely disappeared, and the necrotic fiber segments were replaced by regenerating fiber segments. Detailed electron microscopic studies of rats with passively transferred EAMG, including the localization of antibody and AChR on the postsynaptic membrane, are in preparation (Sakakibara, Engel, and Lindstrom).

Complement Fixation by Anti-AChR Antibody. Since complement fixation by antibodies bound to AChRs on the postsynaptic membrane might contribute to local damage of the membrane and/or be involved in mediating the cellular invasion which occurred, rat antisera to AChR were tested for their ability to bind complement after interaction with solubilized AChR. Table II shows that rat antibodies to eel AChR can bind guinea pig complement after binding to eel AChR. This suggests that the cross-reacting antibodies bound to AChR on the postsynaptic membranes of rat muscle probably also bind complement.

Biochemical Studies. The amount of anti-AChR antibody (10.7×10^{-11} mol) injected into recipient rats was one-third the serum content of a rat in the donor pool. This amount of antibody was double that required to bind the total body content of AChR (5.30×10^{-11} mol) in the recipients. Changes in muscle AChR

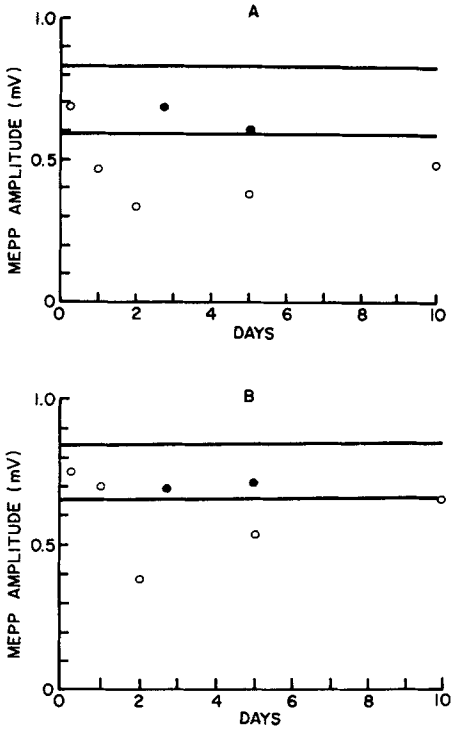

FIG. 4. MEPP amplitude in muscles of rats with passively transferred EAMG. Miniature end-plate potential amplitudes were measured in (A) forearm muscle and (B) diaphragm muscle of rats sacrificed at intervals after receiving 10.7×10^{-11} mol of antibody to rat muscle AChR (○) or 34.2 mg of adjuvant control immunoglobulin (●). Parallel lines delimit control values previously established (14).

content in recipient rats were similar in time-course to those observed during the acute phase of EAMG starting 6 days after immunization with AChR (1). With this amount of antibody, changes in AChR content were somewhat larger and more uniform than observed after immunization. AChR extracted from muscle 6 h after injection of antibody was found to be complexed with antibody (Fig. 6). In addition, the absolute amount of AChR extracted was decreased. By 24 h, the amount of total extractable AChR had decreased to less than half the normal amount (Fig. 6), but there was no change in the amount of AChR complexed with antibody. Between days 3 and 4 the total amount of AChR returned to normal levels. At this time neuromuscular transmission was improving (Figs. 1, 3, and 4). By day 5, when neuromuscular transmission was demonstrable in almost all fibers, the content of AChR exceeded normal amounts (Fig. 6). This suggested that extrajunctional AChR may have been synthesized in fibers which were functionally denervated on days 1–3. Repair synthesis no doubt contributed much to the total as well. Although the AChR content had commenced to decrease toward normal levels by day 6, perhaps in response to reinnervation which appeared to be occurring on days 4 and 5, the absolute amount of AChR was still slightly elevated after 8 days. During days 1–3 about 30% of the AChR remaining in the muscle was complexed with antibody

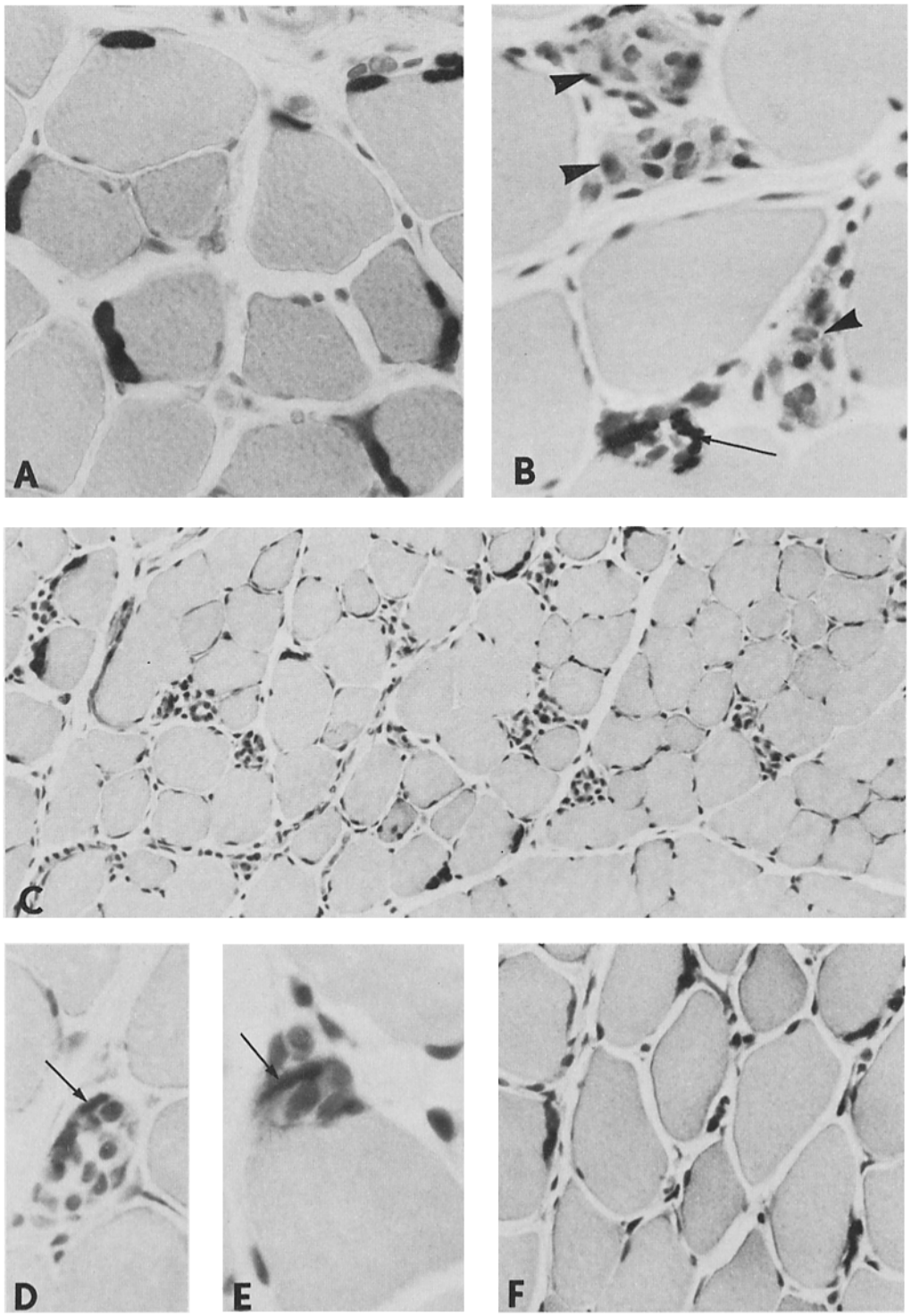
A
B
C
D
E
F

TABLE II
Consumption of Guinea Pig Complement by the Interaction of Eel AChR and Rat Anti-AChR Serum

Moles AChR ($\times 10^{-13}$)	Percent lysis of sensitized SRBC	
	Adjuvant rat serum	Rat anti-AChR serum
568*	0	0
56.8‡	>75	0
5.68	>75	0
0.568	>75	>75
0.0568	>75	>75
0	>75	>75

* Hemolysis was completely inhibited in the presence of a concentration of Triton X-100 equivalent to that contained in this concentration of AChR.

‡ This value represents approximate equivalence to the amount of anti-AChR antibody in the assay.

(Fig. 6). During days 4–8, when the clinical state of the animals was greatly improving, a smaller fraction of the total AChR was complexed with antibody, but the total amount of complexes was increased. By 8 days neither the clinical state nor the total AChR content had returned entirely to normal, and some muscle AChR was still complexed with antibody. The serum content of antibody decreased approximately linearly for the first 3 days (Fig. 6). Thereafter it decreased more slowly. After day 5 more antibody was present complexed with AChR in muscle than was present in serum.

To determine the efficiency of passive transfer, clinical and biochemical signs of EAMG were evaluated in rats injected with graded amounts of antibody to AChR (Fig. 7). The lowest dose of antibody tested (1×10^{-11} mol) induced both clinical signs of EAMG, a decrease in the amount of muscle AChR, and complexing of muscle AChR with antibody. Theoretically this dose would be capable of binding only 17% of the total amount of AChR contained in the musculature of a normal rat, and this amount of antibody represents only 4% of the anti-AChR content of a rat in the donor pool. The decrease in amount of muscle AChR 2 days after passive transfer was almost directly proportional to the dose of antibody injected. The amount of AChR complexed with antibody in the muscles

FIG. 5. Specimens from forelimb digit extensor muscle 6 h (A), 24 h (B), 48 h (C, D, E) and 5 days (F) after passive transfer. The cholinesterase-reactive parts of the end-plates appear as dark, homogeneous spots in these photographs and are easily distinguished during direct observation by their red color as distinct from the blue of stained nuclei. No abnormality is seen at 6 h (A). At 24 h (B) there are necrotic fibers invaded by macrophages (arrowheads), and the end-plate (arrow) is separated from the underlying fiber by invading cells. At 48 h about 10% of the fibers are invaded by macrophages (C). Fragments of cholinesterase-reactive end-plate region (arrow) can still be observed while the underlying fiber region is invaded by macrophages (D). Mononuclear cells invading end-plate (arrow) at higher magnification (E). At 5 days (F) the cellular reaction has subsided. Cholinesterase-reactive regions are of smaller size than at 6 h (compare with A). A and B: × 480; C: × 190; D: × 480; E: × 770; F: 310.

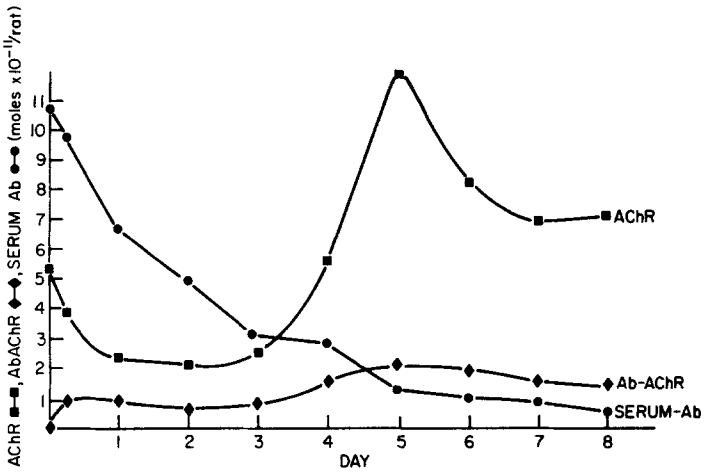

FIG. 6. Changes in content of serum antibody, muscle AChR, and antibody-AChR complexes after injection of antibody to AChR. At time zero rats were injected with 10.7×10^{-11} mol of antibody to AChR. Thereafter, all were tested electromyographically (Fig. 3), and at intervals two rats were sacrificed for biochemical studies. Values for content per rat of serum antibody (●), AChR (■), and antibody-AChR complexes (◆) generally differed less than 10% between the two animals.

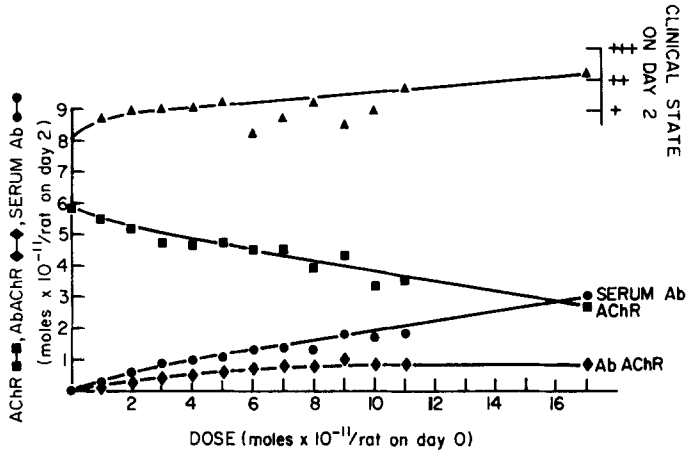

FIG. 7. Dose-response curves for passive transfer of EAMG with increasing amounts of antibody to AChR. Each point is the average of two rats receiving the indicated dose of antibody 2 days before assay.

of recipient rats at day 2 did not increase in direct proportion to the amount of antibody injected, but plateaued at a low level. Doses of 17×10^{-11} mol of antibody were lethal to some rats in 2 days, yet this dose is less than the average content (27×10^{-11} mol) of serum antibody in the rats with chronic EAMG in the donor pool.

To test whether passive transfer of EAMG would trigger a self-sustaining autoimmune response to AChR by presenting antibody-AChR complexes and immunogenic fragments of postsynaptic membrane to immunocompetent cells in the recipients, a group of 10 recipient rats was studied for 72 days after

TABLE III
Absence of Immune Response to AChR at Prolonged Times After Passive Transfer

Group	Initial dose of antibody $\times 10^{-11}$ mol/rat	Antibody at day 21 $\times 10^{-11}$ mol/rat	Antibody at day 72 $\times 10^{-11}$ mol/rat	AChR at day 72 $\times 10^{-11}$ mol/rat
Normal recipients (5)	7.0	0.35 ± 0.17	0.00 ± 0.19	6.23 ± 0.06
Adjuvant immunized recipients (5)	7.0	0.32 ± 0.17	0.043 ± 0.06	6.69

passive transfer of antibody. The rats were given 7×10^{-11} mol of antibody to muscle AChR, and five received additionally 2×10^{10} *B. pertussis* organisms subcutaneously to enhance their immune responsiveness. Although all 10 rats showed the expected signs of EAMG in the days immediately after injection of antibody, none showed signs of a second phase within the next 72 days, no anti-AChR antibody was detectable in the sera after 21 days, and muscle AChR content of recipient rats was normal at 72 days (Table III). Therefore, passive transfer to EAMG did not trigger an autoimmune response.

Discussion

Passive transfer of anti-AChR antibody from rats with chronic EAMG to normal rats caused signs of acute EAMG in the recipients. Features of MG and acute, chronic, and passively transferred EAMG referred to in this paper are compared in Table IV. The most striking similarity between passive and acute EAMG, and the basis of their difference from chronic EAMG and MG, is the cellular invasion of the end-plate region. Phagocytic invasion of the end-plates in rats receiving antibody to AChR suggests that the cells observed at the end-plate during acute EAMG are not primarily lymphocytes sensitized to AChR, but rather nonspecific macrophages responding to antibody bound to AChRs in the postsynaptic membrane, and perhaps also responding to local complement-mediated membrane damage. Electron microscopic studies of both acute (11, 12) and passive EAMG (Sakakibara, Engel, and Lindstrom, manuscript in preparation) clearly show that the cells observed in the end-plate are macrophages. The involvement of macrophages in passive transfer explains the efficiency of the process, since low doses of antibody sufficient to bind only part of the AChRs in the membrane could mark much of the postsynaptic membrane for destruction by the phagocytic cells.

Antibody to AChR might alter neuromuscular transmission by at least five mechanisms: (*a*) antibody may partially or completely inhibit activity of the AChR molecule to which it is bound; (*b*) antibody may cause modulation of the AChR to which it is bound; (*c*) antibody-induced modulation of AChR may cause increased synthesis of AChR; (*d*) antibody bound to AChR may fix complement and cause localized destruction of the postsynaptic membrane; and (*e*) antibody bound to AChR may mark the postsynaptic membrane for destruction by macrophages. Evidence that the first mechanism occurs in EAMG is that antisera from animals with EAMG block AChR activity on muscle (2, 3) and electroplax (4–6) even after inhibition of complement. Decreased ACh sensitiv-

TABLE IV

Comparison Between Features of Passive, Acute, and Chronic EAMG and MG

	Passive EAMG	Acute EAMG	Chronic EAMG	MG
Clinical				
1) Weakness	+	+	+	+
2) Fatigability	+	+	+	+
Electrophysiological				
1) Decrementing electromyogram	+	+	+	+
2) Repair of electromyogram with ACh esterase inhibitor	+	+	+	+
3) Reduced mepp amplitude	+	+	+	+
4) Increased curare sensitivity	+	+	+	+
5) Decreased ACh sensitivity	NT	NT	+	+
6) Reduced muscle action potential amplitude	+	+	−	−
7) Fibers without end-plate potentials	+	+	−	−
8) Reduced resting potential	NT	+	−	−
Histological				
1) Reduced toxin binding at end-plates	NT	NT	+	+
2) Reduced number of postsynaptic folds	−	−	+	+
3) Focal destruction of the postsynaptic membrane	+	+	+	−
4) Phagocytic invasion of end-plate	+	+	−	−
5) Segmental necrosis of some fibers	+	+	−	+
Biochemical				
1) Decreased AChR content	+	+	+	+
2) Transient increased AChR content	+	+	−	−
Immunological				
1) 7S antibodies to muscle AChR	+	+	+	+
2) Complement fixation by anti-AChR antibodies	+	NT	+	+
3) Antibodies not directed at AChR binding site	+	+	+	+
4) Antibodies bind to muscle AChR in vivo	+	+	+	+
5) Antibodies block AChR activity in vitro	+	NT	+	NT
6) Average concentration of antibodies to muscle AChR $>10^{-8}$ M	NT	−	+	+
7) Average concentration of antibodies to muscle AChR $<10^{-8}$ M	NT	+	−	−
8) 19S antibodies to muscle AChR	−	+	−	−
9) Positive skin test response to AChR	NT	+	−	NT

NT, not tested.

ity (3), increased curare sensitivity (14), and small mepps (14) could be explained by either antibody blockage of AChR activity or reduced amounts of AChR. Evidence suggestive of antibody-induced modulation of AChR is the persistence of decreased amounts of AChR in chronic EAMG (1). Increased synthesis of AChR in chronic EAMG is suggested by the observation of substantial amounts of AChR unlabeled by antibody (1). This is striking because passive transfer experiments show that the large excesses of antibody in the serum of rats with chronic EAMG have ready access to the end-plate; so, if AChR there were turning over at the normal low rate (15, 16), then all the AChR should rapidly be

labeled with antibody. In this paper we presented evidence that antibody to AChR in rats with EAMG can fix complement on interaction with soluble eel AChR. Serum from MG patients can also fix complement on binding to particulate eel AChR (17). Thus, it is likely that binding of antibody to AChR in the postsynaptic membrane might also cause fixation of complement and focal membrane destruction. Focal postsynaptic membrane destruction is observed in both acute and chronic EAMG (11, 12). Phagocytic invasion of the end-plates during passive EAMG is striking evidence that antibody to AChR can target the postsynaptic membrane for destruction.

The relative importance of the various mechanisms by which antibody might inhibit neuromuscular transmission varies during the course of EAMG. Clinical and electrophysiological signs approximately parallel AChR amount in the course of passive transfer. The decrease in AChR, in turn, as in acute EAMG, must in large part be related to phagocytic activity at the end-plate which also directly topologically impairs transmission. Before phagocytosis, and at end-plates that are not phagocytized, antibody-induced modulation of AChR and blockage of AChR activity may be important factors in impairing neurotransmission. In chronic EAMG, decreased AChR content per se is probably the principal factor in impairing transmission, and antibody bound to many of the remaining AChRs probably also contributes to impaired transmission (1).

The role of antibodies to AChR in impairing neuromuscular transmission in human MG does not appear to include marking the postsynaptic membrane for destruction by macrophages (18), though small amounts of phagocytic activity might go undetected. As in the case of chronic EAMG, high concentrations of serum immunoglobulin G recognizing muscle AChR are present (6), and these can be used to passively transfer MG to mice (7). In that case, transfer required high doses administered chronically over many days, perhaps because of the limited cross-reaction between human and mouse AChRs, and also perhaps because of limited interaction between human antibody and mouse complement and macrophages. Phagocytic invasion of end-plates was not examined in that experiment. Because the factor terminating phagocytic invasion after acute EAMG is unknown, it is even more difficult to speculate on the regulation of antibody-mediated cellular reactions in MG. Passive transfer of MG between humans appears to occur in the case of neonatal MG. Antibody to AChR apparently transferred across the placenta has been found in the serum of babies suffering from this transient form of MG (6).

We demonstrated that, after the specific degeneration of postsynaptic membrane which occurs in passively transferred EAMG, an autoimmune response is not triggered, despite the presence of antibody-AChR complexes, phagocytic cells, and even a potent adjuvant (Table III). This means that, at least in the case of rats, simply presenting AChR to the immune system, even as multimeric aggregates in membrane with antibody attached, and even with systemic adjuvants, is not sufficient to trigger an autoimmune response. The observation that EAMG intensity increases with the dose of AChR (10) is also consistent with this observation, since, if muscle AChR were immunogenic, then any dose of AChR sufficient to initiate breakdown of the postsynaptic membrane should instead produce equally intense EAMG through autocatalytic immunization with the

breakdown products of the initial attack. Since EAMG is induced by purified rat AChR in amounts smaller than the amounts of AChR affected by passive transfer (1), the critical event allowing induction of EAMG by purified AChR is probably extraction of AChR from the membrane or denaturation of the AChR during purification or emulsification in complete Freund's adjuvant. Similarly, the inductive event in human MG may involve alteration of the structure of the AChR found in muscle or in thymus (6). It is known that antibodies to AChR do not develop subsequent to degeneration of human muscle as occurs in muscular dystrophy or polymyositis (6).

Summary

Passive transfer of experimental autoimmune myasthenia gravis (EAMG) was achieved using the gamma globulin fraction and purified IgG from sera of rats immunized with *Electrophus electricus* (eel) acetylcholine receptor (AChR). This demonstrates the critical role of anti-AChR antibodies in impairing neuromuscular transmission in EAMG. Passive transfer of anti-AChR antibodies from rats with chronic EAMG induced signs of the acute phase of EAMG in normal recipient rats, including invasion of the motor end-plate region by mononuclear inflammatory cells. Clinical, electrophysiological, histological, and biochemical signs of acute EAMG were observed by 24 h after antibody transfer. Recipient rats developed profound weakness and fatigability, and the posture characteristic of EAMG. Striking weight loss was attributable to dehydration. Recipient rats showed large decreases in amplitude of muscle responses to motor nerve stimulation, and repetitive nerve stimulation induced characteristic decrementing responses. End-plate potentials were not detectable in many muscle fibers, and the amplitudes of miniature end-plate potentials were reduced in the others. Passively transferred EAMG more severely affected the forearm muscles than diaphragm muscles, though neuromuscular transmission was impaired and curare sensitivity was increased in both muscles. Some AChR extracted from the muscles of rats with passively transferred EAMG was found to be complexed with antibody, and the total yield of AChR per rat was decreased. The quantitative decrease in AChR approximately paralleled in time the course of clinical and electrophysiological signs. The amount of AChR increased to normal levels and beyond at the time neuromuscular transmission was improving. The excess of AChR extractable from muscle as the serum antibody level decreased probably represented extrajunctional receptors formed in response to functional denervation caused by phagocytosis of the postsynaptic membrane by macrophages. The amount of antibody required to passively transfer EAMG was less than required to bind all AChR molecules in a rat's musculature. The effectiveness of small amounts of antibody was probably amplified by the activation of complement and by the destruction of large areas of postsynaptic membrane by phagocytic cells. A self-sustaining autoimmune response to AChR was not provoked in animals with passively transferred EAMG.

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