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BIOLOGY OF MYASTHENIA GRAVIS

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INTRODUCTION

The chronic muscular weakness and fatigability characteristic of myasthenia gravis (MG) results from defective neuromuscular transmission caused by an autoimmune response to acetylcholine receptors (AChR) in skeletal muscle. The initial direct evidence for this long held suspicion (1) was the observation that rabbits immunized with purified AChR developed MG-like weakness (2). Subsequent studies of experimental autoimmune myasthenia gravis (EAMG) have substantially illuminated the complex pathological mechanisms by which the aberrant autoimmune response to AChR in MG impairs neuromuscular transmission. Neither the cause of the autoimmune response in MG nor a cure is known. However, knowledge of the pathological mechanisms is being used to improve both diagnosis and therapy.

Because it is the binding of antibodies to parts of the AChR molecule that begins the pathology at the endplate, and because properties of the AChR molecule are as critical as properties of the immune system in determining the ultimate effects of this antibody binding, we first briefly review AChR structure, function, and metabolism. The structure of AChR from electric organs is best understood, the metabolism of AChR in noninnervated muscle cells is best understood, and AChR function has been best studied in intact muscle cells. We take the point of view that AChR structure, metabo-

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lism, and function are more or less universal, with allowances for the effects of innervation during development and interspecies antigenic variation. In the following two sections we review pathological mechanisms impairing neuromuscular transmission in EAMG and MG. We take the point of view that the pathological mechanisms at the endplate in chronic EAMG and MG are essentially identical, and that muscular weakness results primarily from loss of AChR. With this background, clinical features of MG are reviewed in the next section. Then we consider diagnosis and therapy of MG, and particularly new trends in diagnosis and therapy, in terms of the pathological mechanisms acting at the endplate.

THE ACHR PROTEIN

The AChR is an integral membrane protein. It contains acetylcholine binding sites (3) that regulate, through a change in conformation (4, 5), the opening of a cation-specific membrane channel which is also an integral part of the molecule (6, 7). AChR purified from the electric organs of the marine ray *Torpedo californica* contains four subunits (3, 8) in the mole ratio $\alpha_2\beta\gamma\delta$ (9). The apparent molecular weights of α, β, γ, and δ approximate 38, 50, 57, and 64 $\times$ 10^3, respectively (3, 8, 9). Torpedo AChR normally exists as dimers formed by disulfide bonds between δ subunits (10). The α subunit of AChR from torpedo can be affinity labeled with cholinergic analogues (3, 11, 16) showing that α subunits compose part or all of the acetylcholine binding site. The functions of the other subunits are unknown. All four subunits are acidic glycopeptides (9, 12). AChR from the electric organs of the fresh water fish *Electrophorus electricus* can be prepared under conditions which preserve four subunits (13). AChR from *Electrophorus* has α' subunits which can be specifically affinity labeled with cholinergic analogues (3). All four of its subunits, α', β', γ', and δ' can be shown to correspond immunochemically to the four subunits composing *Torpedo* AChR (13, 14). Rat muscle AChR has α_1, α_2, β, γ, and δ subunits (15). In AChR from rat muscle both α_1 and α_2 subunits have similar peptide maps and both (15) can be affinity labeled. In AChR from fetal calf muscle affinity labeling of only a single molecular weight component occurs (14), as in the case of AChR from electric organs (3). However, even in *Torpedo* electric organ AChR the situation is complicated by the observation that the two α subunits in the molecule are functionally nonidentical, in that only half can be affinity labeled with either agonists or antagonists (16), whereas α-bungarotoxin (αBGT) appears to be able to bind to both. AChR from rat, human (17), and fetal calf (14) contains four sets of antigenic determinants corresponding to the four subunits of *Torpedo* AChR, but it has not yet been shown that, as in the case of *Electrophorus* AChR, they

correspond to the four peptides of similar molecular weights observed in the purified AChR. However, it seems likely that AChRs from electric organs and muscle will turn out to be very similar in structure. In vivo cross-reaction of antisubunit sera (14, 17) suggests that parts of all four subunits are exposed on the external surface of the postsynaptic membrane. By electron microscopy (18, 19) AChRs viewed from the end appear like lumpy doughnuts with negatively staining centers. Pits are observed in the center of the AChR molecules split by freeze fracture of AChR-rich membranes (19). This suggests that a transmembrane pore may run through the center of the molecule, but it is not known whether this pore corresponds to the ion channel. By electron microscopy (21) and X-ray diffraction (22), AChRs viewed from the side appear somewhat mushroom-like, extending more on the extracellular than intracellular surface of the membrane. Cytoplasmic filaments within the cell and basal lamina outside may be associated with aggregations of AChR (19, 20).

AChRs in the postsynaptic membrane of rat muscle stimulated with acetylcholine produce an elementary conductance event in the form of a square pulse of 34pS amplitude and 1 msec duration (23). Although one acetylcholine molecule appears to be able to activate an AChR, two acetylcholine molecules appear much more effective (24). Under normal conditions, activation of a single AChR would permit passage of about 5×10^4 ions (25). On continued exposure to agonists, AChR desensitize. The ion channel of desensitized AChR is closed, and the binding affinity for agonists appears to increase greatly (26, 27). Desensitization is reversible. The role of desensitization in normal neuromuscular transmission is not clear.

AChR metabolism has been recently reviewed (28). In cell culture, AChRs are synthesized (presumably on rough endoplasmic reticulum) and assembled to the point where they can bind ^{125}I-αBGT in less than 15 min. Completion of their assembly appears to require glycosylation. AChR capable of binding αBGT are first observed in the Golgi apparatus with their ligand binding sites on the interior of closed membrane vesicles. Two to four hours are required before AChR are finally incorporated in the plasma membrane. In denervated muscle, like primary muscle cultures, AChR are incorporated and maintained in large and small patches all over the surface of the cell, whereas in innervated muscle AChR are concentrated at the tips of folds in the postsynaptic membrane. Extrajunctional AChR of denervated muscle are present in tenfold or greater amounts per cell than are junctional AChR of innervated muscle. But extrajunctional AChR are degraded almost tenfold faster than junctional AChR [extrajunctional $T_{1/2} \approx 17$ hr versus junctional $T_{1/2} \approx 150$ hr (48)]. Degradation of AChR requires energy, and appears to involve internalization into secondary lyso-

zomes followed by proteolytic digestion and finally release of the resulting amino acids from the cells.

PATHOLOGICAL MECHANISMS IN EAMG

EAMG has recently been reviewed in some detail (29), but here is dealt with succinctly. EAMG is observed in both acute and chronic forms, but the chronic form appears most relevant to what occurs in MG.

Lewis rats immunized with AChR protein in complete Freund's adjuvant and given pertussis vaccine at other sites as additional adjuvant develop an acute form of EAMG 8–10 days after immunization (30). If acute EAMG is not severe enough to be lethal, it is transient and the rats appear to have regained their normal strength in 2–3 days. The characteristic feature of acute EAMG is a massive phagocytic invasion of the endplates (31). At this early time in the immune response, serum anti-AChR concentration is low and few AChR are labeled with antibodies (32). However, the amplification effect of the phagocytes results in destruction of large areas of postsynaptic membrane and much of the muscle's AChR content (32). This causes functional denervation of many fibers (33) which respond by synthesizing extrajunctional AChR (32). AChR content and transmission return to normal levels within a few days. The acute phase of weakness is not observed if the additional adjuvant pertussis is omitted. Thus, this cell-mediated chaos may be an artifact of the intense use of adjuvants, and is certainly not a necessary prerequisite for observing chronic EAMG. It cannot be excluded, however, that a less intensive phagocytic response may occur without the use of pertussis. What limits the duration of the phagocytic attack is not known with certainty.

A phagocytic attack on the postsynaptic membrane which closely resembles that in acute EAMG occurs after injection of anti-AChR antibody into normal rats (34). In this case, and presumably in the case of acute EAMG, the phagocytic attack (35) depends not only on the labeling of AChR on the postsynaptic membrane with anti-AChR antibodies (34), but also on the subsequent deposition of complement (36). If rats are depleted of the C-3 component of complement, passive transfer of anti-AChR antibody results in labeling most of their muscle AChR with antibody, but phagocytic invasion does not occur, and neuromuscular transmission is not detectably impaired (36). This shows that the phagocytes are passively immunized and must detect both bound antibody and bound C-3 before they can attack the postsynaptic membrane. It also shows that the direct effects of bound antibody on the function of muscle AChR are small, since labeling of 67% of the AChR with antibody did not detectably impair transmission. However, it must be remembered that these rats contained normal amounts of

AChR organized at the tips of normal postsynaptic membrane folds, and transmission was protected by a large safety factor. In rats with chronic EAMG, AChR content is reduced (32, 37), postsynaptic membrane structure is disorganized (31, 37), and the small but measurable decrease in AChR channel opening time and conductance due to bound antibody (38) might then compromise transmission to a much greater degree.

Chronic EAMG is characterized by simplified postsynaptic membrane structure in the absence of phagocytes (31, 39), high concentrations of anti-AChR antibodies in serum (40), binding of antibodies to most AChR in muscle (32), and reduction of AChR content to about 30% of normal (32, 41). In chronic EAMG (32, 41) as in human MG (37, 42), loss of AChR appears to be the primary factor impairing transmission. This directly reduces the acetylcholine sensitivity of the membrane (43). Loss of acetylcholine sensitivity alone, as produced, for example, by injection of αBGT to competitively inhibit AChR (44), is sufficient to produce the weakness, fatigability, and decrementing electromyogram response characteristic of MG and EAMG. Direct effects of bound antibody on AChR function (36, 38) and disruption of postsynaptic membrane structure (31, 37) appear to be of secondary importance in reducing acetylcholine sensitivity. Because phagocytic cells are not observed, and the onset of chronic EAMG is coincident with the peak of antibody response to AChR (40), it is thought that AChR loss in chronic EAMG is mediated by antibody. One mechanism by which this occurs is antibody-dependent complement-mediated focal lysis. By electron microscopy, focal lysis of the postsynaptic membrane is observed (31, 35, 39). Membrane fragments containing AChR, antibody, and C-3 are observed in the intersynaptic cleft (37, 45). Another mechanism by which AChR loss occurs in EAMG is antigenic modulation. This does not depend on complement, but is an increase in the rate of AChR destruction (38) due to cross-linking of AChR by anti-AChR antibodies (41). Bivalent anti-AChR antibodies, but not monovalent FAb, cross-link (41) AChR, cause rapid aggregation in the plane of the membrane and internalization (46). This is an energy- (38), temperature- (38), and lysozomal enzyme- (47, 48) dependent process, like the normal process of AChR destruction (28, 48). Antigenic modulation of AChR occurs both in tissue culture (38), organ culture (49), and in muscle removed from rats with EAMG (47). The rate of destruction of both junctional (47, 48) and extrajunctional (47, 49) AChR is increased two to three fold over normal. It has been speculated that the rate-limiting step in the destruction of AChR is internalization by endocytosis (41). In the absence of a compensatory increase in AChR synthesis in rats with EAMG, a two to three fold increase in the rate of AChR destruction due to antigenic modulation is sufficient to account for the observed two thirds loss of AChR in these rats (41).

However, if in rats with EAMG, AChR synthesis were increased from the low rate characteristic of innervated muscle, the role of antibody-dependent complement-mediated focal lysis might be larger to account for the observed AChR losses.

Antigenic determinants that depend on the native structure of the AChR molecule appear to be the most immunogenic and myasthenogenic (14, 17). These appear to be located primarily on the α subunit (50). However, immunization with large amounts of any of the four denatured subunits of *Torpedo* AChR can cause EAMG (14, 17). Denatured α and δ subunits are most effective at provoking antibodies that cross-react with rat AChR and most effective at provoking clinical EAMG. These results suggest that any antibody directed at the extracellular surface of the AChR molecule should be able to cause EAMG. Neither of the mechanisms thought to cause AChR loss (antigenic modulation and complement-mediated focal lysis) should depend on where antibody binds to the AChR molecule. The direct effects of bound antibody on AChR channel opening time and conductance are small (38), and antibodies bound to most of the AChR in a normal rat's muscles do not compromise transmission (36). Few (51) or none (52) of the antibodies to AChR bind at sites close enough to the acetycholine binding site to specifically inhibit the binding of toxin, much less acetylcholine. Together these results suggest that there is no single "myasthenogenic" determinant on the AChR molecule at its acetylcholine binding site or elsewhere. However, there have been some interesting experiments thought to demonstrate the existence of a single myasthenogenic antigen on AChR (53), but these can also be explained in other ways (29). There are some effects of antibody specificity (54, 55). For example, in EAMG the majority of antibodies to the electric organ AChR used as immunogen do not cross-react with muscle AChR; only the 1 or 2% that cross-react with muscle AChR are pathologically relevant (40). In both EAMG and MG, antibodies directed at antigenic determinants on the intracellular surface of the molecule would be detected by the normal assays using detergent-solubilized ^{125}I-α-bungarotoxin labeled AChR as antigen, but would be pathologically irrelevant because they could not bind in vivo.

PATHOLOGICAL MECHANISMS IN MG

Decreased acetylcholine sensitivity of muscle in MG patients appears to be the final common mechanism by which various factors act to produce muscular weakness and fatigability. The amount of acetylcholine released by the nerve at myasthenic endplates is at least as large as normal (56), but endplate potentials are smaller (57) due to decreased acetylcholine sensitivity of the postsynaptic membrane (58). The decrease in sensitivity does

not depend much on the direct effects of antibodies on AChR channel opening time or conductance (57, 59). As in chronic EAMG, the decreased sensitivity results primarily from loss of AChR. At an endplate in human intercostal muscle an activated nerve ending releases about 60 quanta of acetylcholine (60), each containing about 10^4 molecules of acetylcholine (61). Normally a quantum of acetylcholine activates 1500 AChR, whereas in the average MG patient it activates 500 (57). In the average MG patient, the AChR content of intercostal muscles is 36% of normal (42), or about 2×10^6 AChR molecules per endplate (62). An average of 54% of the AChR that remain have antibodies bound (42). The acetylcholine sensitivity is directly proportional to the amount of AChR remaining (37, 42).

The pathological mechanisms by which AChR content and acetylcholine sensitivity is reduced in MG appear to be the same in MG and chronic EAMG. The animal model appears to differ from MG primarily in that the autoimmune response to AChR in EAMG results from the small but significant cross-reaction of antibodies to electric organ AChR with muscle AChR (40, 54), and the autoimmune response parallels the course of the response to the foreign immunogen (40, 63), whereas in MG antibodies appear to be directed primarily at human AChR (54) and arise and are sustained by an endogenous process which is not understood.

Postsynaptic membrane morphology is simplified in MG (64). This would be expected to decrease acetylcholine sensitivity by misorienting specific sites of acetylcholine release (65) with respect to specific concentrations of AChR at the tips of postsynaptic membrane folds (65a). Phagocytic invasion of endplates is not observed, suggesting that the damage is done by antibody-mediated mechanisms (39). Antibodies are bound to most of the AChR extracted from the muscle of MG patients (42), and bound antibody can be localized in situ using peroxidase conjugated to protein A (66). The C-3 (66) and C-9 (67) components of complement can also be localized by antibody-peroxidase methods. The presence of C-9 indicates that the full complement sequence has been activated. Membrane fragments containing AChR, antibody, and complement are observed in the synaptic cleft. This suggests that antibody-dependent complement-mediated focal lysis is probably largely responsible for the morphological alterations in the postsynaptic membrane and in part responsible for the observed loss in AChR.

Probably the largest factor responsible for reducing the AChR content of muscle in MG is antigenic modulation. Sera from MG patients cause an increase in degradation rate of AChR in rat muscle cells in culture (68, 69) by cross-linking these AChR (70). The mechanism of AChR destruction appears to involve internalization and lysozomal degradation, as in the case of EAMG (38, 41, 47, 49).

Serum anti-AChR antibodies are detectable in at least 87% of patients otherwise thought to have MG (71, 72). Concentration varies from barely detectable levels of about 0.62×10^{-9}M to $> 1000 \times 10^{-9}$M. Although patients with only ocular weakness have significantly lower concentrations, among patients with generalized MG, absolute concentration of anti-AChR does not correlate closely with disease severity (71). However, as is discussed in subsequent sections, serial measures of anti-AChR antibody concentrations within individuals do correlate well with severity. Other evidence for the importance of anti-AChR antibody in MG includes the association between neonatal MG and the transplacental transfer of anti-AChR antibody (73), passive transfer of muscular weakness to mice using IgG from MG patients (74), and the observation of clinical improvement in MG patients whose anti-AChR concentration was directly decreased by means of plasmapheresis (75, 76).

The source of immune stimulation or antibody synthesis in MG is unknown. The abnormal frequencies of some HLA determinants in MG patients (77) and the increased frequency of some other autoimmune diseases in MG patients (1) suggest that some MG patients may have had an abnormal susceptibility to autoimmune response. Treatment of some patients suffering from rheumatoid arthritis with penicillamine causes a transient form of MG that remits after the penicillamine is withdrawn (82–84), but it is unknown whether penicillamine acts by modifying the immune response or by modifying AChR to make them immunogenic. The immunogen that normally provokes MG is unknown, but the specificities of anti-AChR in the sera of MG patients suggest that it at least closely resembles human AChR (54) and might have some features of extrajunctional AChR (78). Muscle-like cells in the thymus have been suspected as the source of this AChR (79), but there is no direct evidence. Anti-AChR antibody synthesis in cell culture has been detected using cells from the thymus (80, 81) and peripheral B lymphocytes (C. Tsoukas, J. Vaughn, M. Seybold, and J. Lindstrom, unpublished) of some patients. But little is known about the origin or control of anti-AChR synthesis in MG. It is unlikely that a large fraction of anti-AChR is synthesized in the thymus, since thymectomy does not produce a dramatic decline in anti-AChR concentration (85).

When considering pathological mechanisms in MG, a factor not usually considered in studies of EAMG must be taken into account. This factor is that MG patients are individuals. Unlike young, female, inbred rats immunized with electric organ AChR, patients differ in age, sex, genetic background, and disease history. In addition to certainly differing in age, sex, and HLA type, MG patients might differ in the AChR immunogen or cause of the autoimmune response, regulation of the immune response, anti-AChR antibody subclass or specificity, rates of AChR metabolism, and

many other factors. All these factors could produce variations between patients without altering the fundamental similarities in the mechanisms by which their neuromuscular transmission is impaired.

CLINICAL FEATURES OF MG

MG is characterized clinically by abnormal fatigability and weakness of voluntary striated muscle. The weakness is worsened by exercise and partially relieved by rest. Usually muscles supplied by the cranial nerves are the first and most severely affected, with resultant drooping eyelids, double vision, lack of facial expression, slurred speech and difficult swallowing, followed later by weakness of neck flexion, proximal extremity muscles, and the muscles of respiration. Why some muscles are more affected than others is not known. It may be that the more susceptible muscles have lower safety factors for transmission as a result of lower amounts of AChR or other quantitative or morphological factors. For example, in mice, at least, extraocular muscles have fewer and smaller junctional folds than do diaphragm muscles (86). After prolonged MG an atrophic process may set in in some muscles, probably due to chronic denervation. This may eventually become irreversible (87). The natural history of MG is one of progression for 2 to 5 years (88), although some cases never develop more than mild weakness. Spontaneous remissions occur, but are usually short-lived (89). In a recent series (90) of 80 MG patients who received symptomatic treatment without any immunologic intervention in the disease process, the 10-year mortality was 40%, and the average time to death after onset of MG was seven years.

The full blown picture of MG is easily recognized clinically, but in early cases the diagnosis is often difficult because the major symptom may be fatigability, and weakness may be limited to a few or even single muscles (91). The standard objective diagnostic technique for MG has been electromyography. In MG, a rapid decline in amplitude of successive compound action potentials is usually evoked from affected muscles upon supramaximal electrical stimulation of their motor nerves at rates of 4–5/sec (92). This decrementing electromyogram can be returned to its normal constant amplitude by injecting acetylcholinesterase inhibitors. A new approach to diagnosis of MG is detection of antibodies to AChR in serum. This is both sensitive and objective. Antibodies to human AChR are detectable in approximately 90% of patients otherwise thought to have MG, and are not detected in other neuromuscular or autoimmune diseases (71). Antibodies to AChR are not detected in congenital MG (93), an inappropriately named disorder which must be distinguished from acquired MG, which it resembles clinically (94). Half of congenital MG cases are

familial and presumably are caused by a mutation at some step in neuromuscular transmission. The immunosuppresive therapies effective for MG obviously would not be beneficial for these patients.

The prevalence of MG is about one in 20,000 population, and the age and sex distribution is bimodal (95). Females predominate over males (2:1) in the larger, first peak of incidence (20 to 40 years), but the sex ratio is reversed in the second peak (50 to 70 years). In Caucasions there is an increased frequency of the HLA-B8 (96) and HLA-DRw3 (97) (which exists in strong linkage disequilibrium with HLA-8) histocompatibility antigens in the younger MG age group, and of HLA-A2 and HLA-A3 (77, 98) in the older population of MG patients. Ten percent of MG patients have thymoma; of these, 72% occur in the over-40 age group (99, 100). Nine percent of MG cases occur in juveniles (95), and 17% in patients over 65 years, where the prognosis is exceptionally poor (101).

Neonatal MG is a transient syndrome of weakness occurring in 12% of infants born to myasthenic mothers, and is probably caused by the facilitated transfer of IgG from the maternal into the fetal circulation during pregnancy (102).

There is only a rough correlation between the anti-AChR titer and the severity of the patient's clinical symptoms (71, 103). Significantly lower anti-AChR titers are found in patients with only ocular symptoms, and higher titers are present in patients with late, severe MG and with thymoma (71, 103), a group in which the disease also is generally more severe (99). Higher titers are present in patients with greater than 50% impairment of neuromuscular transmission as measured by the electromyographic decremental response (104), and in nonthymomatous HLA-B8 positive patients (97). About 13% of MG patients do not have detectable serum anti-AChR; they are usually patients in remission, or with only ocular or mild generalized MG (71, 103). Relative changes in anti-AChR titer in a given patient correlate well with changes in clinical severity (76), but additional factors such as emotional stress and hormonal changes also undoubtedly influence a patient's clinical status (88, 105).

In addition to the 10% incidence of thymoma, the thymus shows a histological abnormality in a further 72% of patients in that it contains numerous medullary germinal centers (106), a finding which correlates strongly with presence of HLA-B8 (107). Since germinal centers appear in lymphoid tissues in response to antigenic stimulation, and are centers of antigenic processing and antibody production (108), it may well be that the autoimmune process leading to MG begins in the thymus. Indeed, AChR is expressed on striated muscle-like cells, which can be cultured from the thymus gland (79, 109), and cultured thymocytes can produce antibodies to AChR (80). Thus a beneficial clinical response to thymectomy in MG

might be expected due to removal of a source of antigenic stimulation and autoantibody production. However, although some reports indicate that anti-AChR titers are lower in thymectomized than in nonthymectomized MG patients (103, 110), study of individual patients before and after thymectomy has not always shown a fall in anti-AChR titers (81, 85, 111).

Additional evidence for deranged immune function in MG is provided by the frequent appearance of other autoantibodies and the occurrence of MG together with other autoimmune diseases in the same patient. Hypocomplementemia (112) and circulating immune complexes have also been described (113, 114). An autoantibody reacting against skeletal muscle in striated pattern, possibly at the level of the sarcoplasmic reticulum (115), is present in the serum of 95% of thymomatous MG patients and in 10% of those without thymomas (116). The antimuscle antibody crossreacts with cardiac muscle and thymic epithelial cell cytoplasm (117). Inflammatory cardiac lesions occur in MG, almost always in association with thymoma (118). Antinuclear, antithyroid and anti-IgM autoantibodies also occur with increased frequency in MG patients (119–122). Thyrotoxicosis, an autoimmune disease caused by antibody to TSH receptors, is found in 5% of MG patients, an incidence 50 times greater than in the general population (123). Hashimoto's thyroiditis (122), pernicious anemia (101), idiopathic inflammatory myositis (124), arthritis (125), phemphigus (126), and systemic lupus erythematosus (127, 128) all occur with increased frequency in MG. Thirty cases of MG induced by D-penicillamine are now known, 28 of them in patients who were being treated for rheumatoid arthritis (129). D-penicillamine is a potent inducer of autoantibodies and autoimmune diseases. Both antistriational and anti-AChR antibodies have been reported in patients receiving D-penicillamine therapy (83, 84, 130). MG accompanied by antibodies to muscle has also been induced by trimethodione (131), another drug known to promote the development of autoimmunity.

The frequent association of related autoimmune phenomena in MG, the occasional familial occurrence of MG, and the increased frequency of certain histocompatibility antigens in MG suggest that there may be a genetic susceptibility to the development of MG. Nonthymectomized noncorticosteroid-treated MG patients have significantly increased titers of antibody toward cytomegalovirus (132), perhaps reflecting a defective immune elimination of this agent. Susceptible individuals might have a defect in immunoregulation, for example, in dampening autoimmune responses which might naturally arise during the course of infectious diseases, drug therapy, or trauma. One might expect to find defective suppressor T-lymphocyte function in MG patients, which in fact has been demonstrated in a small group (133).

THERAPY OF MG

Anticholinesterase Medications

MG patients are a heterogenous population. The most significant points of difference between patients in planning therapy are age of onset, presence of thymoma, duration and severity of symptoms, and history of previous treatment. The first form of therapy to be carried out is generally symptomatic treatment with drugs that inhibit acetylcholinesterase. These drugs compensate in part for the loss of AChR in MG by increasing the local concentration and duration of acetylcholine. The most widely used anticholinesterase is pyridostigmine, which has a longer effect (3 to 6 hr) and therefore smoother action than neostigmine (2 to 4 hr), an effective but less convenient medication (134). MG patients stabilized on widely different doses of oral pyridostigmine bromide (60 to 660 mg/day) showed therapeutic plasma levels within a relatively narrow range (20 to 60 ng/mg) (135), an effect which is largely due to a widely variable gastrointestinal absorption of pyridostigmine among individuals (136). In patients with mild generalized MG, ocular MG, or in partial remission, therapy with anticholinesterase medication alone may provide adequate relief of symptoms (137). Acute overdoses of antiesterase drugs cause an acute impairment of neuromuscular transmission in normal animals, probably by causing accumulation of desensitized AChR. Chronic exposure of normal animals to excesses of antiesterase drugs results in disruption of postsynaptic membrane structure superficially resembling that in MG. However, the mechanism of the disruption appears to involve changes in ion gradients caused by overactivation of AChR (138, 139).

Thymectomy

It is evident that any therapy attempting to alter the underlying basis for MG must be directed at the immune system. Because of the presence of thymic lesions in MG, thymectomy had been established empirically as a valuable therapeutic modality long before MG was recognized to be an autoimmune disease (140, 141). In the case of nonthymomatous MG it was soon established that increasing numbers of germinal centers in the thymus gland removed at thymectomy were associated with a greatly prolonged time until onset of clinical remission (142, 143) (defined as absence of symptoms without anticholinesterase drug therapy), although by 10 years after surgery all patients had attained the same remission rate (143). In any case, the number of remissions gradually increase with time postthymectomy; 50% of them come two or more years after surgery (144). These findings led to the concept that autosensitization toward AChR was occurring in the thymus, from which sensitized lymphocytes might seed other

lymphoid organs to establish enough autoantibody production to produce clinical disease. Early thymectomy might interrupt this process before large numbers of autosensitized cells had been produced. Remission might be delayed following late thymectomy because large numbers of long-lived lymphocytes sensitized toward AChR had already established themselves in extrathymic lymphoid tissue. Indeed, Genkins et al (145) found the numbers of thymic germinal centers increased with increasing duration and severity of the MG, and the response to thymectomy during the first year of the disease to produce better results than had been previously reported, the percentage of remissions reaching 40% at 5 years postthymectomy. Recent results suggest that patients with high numbers of circulating T-lymphocytes and evidence of a high level of T-lymphocyte function have the best response to thymectomy (146).

Certainly when compared to matched patients treated with anticholinesterase medications alone, thymectomy in nonthymomatous MG is beneficial: In a study by Buckingham et al (90), at 20 years follow-up 34% of 80 thymectomized patients were in remission compared to 8% of a medically treated matched control group. Five-year survival in the surgically and medically treated groups was 90% and 70%, respectively. The response to thymectomy in patients with thymoma has been distinctly worse. In a recent series of 141 patients the remission rate was only 7% and the five-year survival was 60% (99).

Thirty percent of all patients with thymoma develop MG (147), which is strongly associated with the presence of hyperplastic germinal center formation in non-neoplastic thymic tissue adjacent to the thymoma (148). Oddly enough, out of a recent series of 72 patients with thymoma, 7 of them developed MG three years (mean) *after* complete thymectomy (149). In such cases it is possible that subclinical autosensitization of AChR had already occurred prior to surgery, and then had become clinically manifest at a later date through the action of additional factors, such as in a thymomatous MG patient in whom MG was precipitated by a wasp sting (76).

Although early thymectomy has been widely embraced as the immunologic intervention of choice in nonthymomatous patients with generalized MG in the 20- to 50-year age group, in the group with onset over the age of 50 its therapeutic effect is controversial. As early as 1949 and 1953, Keynes (150) and Schwab & Leland (151) reported a less satisfying therapeutic response to thymectomy in the older age group. This impression has been sustained in some later series (152, 153), and Perlo et al (154) found marked thymic atrophy or absence of thymic tissue in 22 MG patients over the age of 60. Identical findings were present in 6 normal controls. On the other hand, in a recent article by Slater et al (155), the

postthymectomy remission rates (23 to 40% at 5 years) and the electromyographic improvement rates were as good in the MG group over 40 years as in those under 50 years of age. Taken together with the findings of male predominance and an association with different histocompatibility antigens in the 50–70 year MG population as compared to the 20–40 year MG population, as well as an increased frequency of cell-mediated immune responsiveness to AChR (156), a worse response to thymectomy in the older MG population could reflect the operation of somewhat different pathophysiological mechanisms in the two age groups. This could be particularly true during the initiation of the disease process, since the proximate cause in both cases is the interaction of antibody with AChR in the muscle membrane.

The thymomatous MG population represents yet a third MG group which has its own unique features which may indicate special initiating pathophysiological circumstances. As Keesey (157) pointed out in 14 cases, HLA-B8 is underrepresented in this group. The constellation of a distinctive occurrence of histocompatibility markers, the almost universal presence of antibody to muscle striations (116), a poor response to thymectomy (99), but an exceptionally good response to corticosteroid therapy (158) and plasmapheresis (159), high anti-AChR antibody titers (71), and the presence of thymoma define another MG subgroup which may warrant separate consideration.

Corticosteroid Therapy

Patients with ocular or generalized MG with significantly impaired function despite optimal therapy with cholinesterase inhibitors and thymectomy where indicated are candidates for corticosteroid therapy. Prednisone is generally administered, although some patients may have more favorable response with dexamethasone or corticotropin (160). Prednisone is initiated in a dose of 60 to 80 mg daily. Once a good clinical response is obtained, it is tapered to an alternate-day basis and further reduced as far as possible to minimize the inevitable dose-dependent side effects which occur with long-term corticosteroid therapy. Up to 92% of MG patients will have a positive clinical response to prednisone therapy, and 32% achieve complete clinical remission (161). Paradoxically, in about one half of MG patients there is a transient exacerbation of weakness during the month after initiation of prednisone treatment. The prednisone-induced weakness becomes severe in 5% of patients, and forces discontinuation of the drug in some of them. Tapering the dose of prednisone frequently leads to relapse of MG, which may not remit when the patient is returned to his previous level of medication. Thirty mg every other day is a lowest effective dosage for many patients (101) and few are ever able to discontinue prednisone altogether.

Prednisone treatment reduces IgG synthesis in man (162), and anti-AChR titers have been shown to decline rapidly with the institution of prednisone therapy (105). However, the rapid improvement manifested by some patients receiving prednisone therapy [within two days, ref. (161)], and the prednisone-induced exacerbation of weakness seen in others, indicates that the effects of corticosteroids in MG are complex and not simply to be explained by a reduction of antibody synthesis. Anti-inflammatory effects of corticosteroids could conceivably account for the rapid return of strength in some MG patients. Patten (163) reported evidence from in vitro studies for an antagonism between corticosteroids and anticholinesterase medications at the motor endplate, which would explain, in part, corticosteroid-induced weakness in MG. Corticosteroids do not, however, appear to have any direct pharmacological effect on neuromuscular transmission (164).

Cytotoxic Drugs

Of the many cytotoxic, immunosuppressive drugs, only azathioprine has been widely used in MG. Azathioprine is an antimetabolite which, after hepatic conversion into its active form, 6-mercaptopurine, selectively kills dividing cells by interfering with inosinic acid metabolism (165). It inhibits immunoglobulin synthesis in man (166), and reduces anti-AChR titers when administered to MG patients (103, 167). Azathioprine can produce therapeutic responses in MG comparable to prednisone. In one recent series (168) 92% of 53 patients were improved. Unimproved patients had all been treated for less than 18 months, and every patient treated for more than 5 years achieved at least a 50% increase in strength. The onset of clinical improvement is slow, never occurring before a minimum of 3 months of continuous therapy, so it is recommended that azathioprine best be used in combination with other immunologic treatment methods such as thymectomy, corticosteroids, or plasmapheresis (169).

Plasmapheresis

Plasmaphersis therapy for autoantibody-mediated diseases of solid organs began at Hammersmith Hospital in London when C. M. Lockwood and his colleagues successfully applied it to the treatment of Goodpasture's syndrome (170), a disease of fulminating renal failure and pulmonary hemorrhages caused by an antiglomerular basement membrane (GBM) antibody which cross-reacts with pulmonary basement membrane. Intensive, short-term plasmapheresis is the appropriate regimen in bringing a severe but self-limited illness such as Goodpasture's syndrome under control, but this approach has not been adequate in treating MG, where there is sustained production of autoantibody over many years with little tendency to sponta-

neous remission. Patients treated by Newsome-Davis and his colleagues (159) were able to restore their titer of circulating antibody to AChR halfway back to the pretreatment value within only 9 days after finishing their course of intensive plasmapheresis, regardless of whether or not they received concomitant immunosuppressive drug therapy with prednisone, azathioprine, or cyclophosphamide. When the titer of anti-AChR antibody finally returned to its preexchange value, as it did in the majority of patients, the patients had clinically relapsed to their pretreatment level of functioning (75). In another study (171) plasmapheresis produced no additional lowering of anti-AChR antibody titers beyond the 20% reduction in titer found after 5 months of therapy in a control group receiving immunosuppressive drugs alone.

In our series (172) of 32 consecutive MG patients treated with an extended series of weekly plasmapheresis and immunosuppressive drug therapy, 19 attained a stable improvement, 7 suffered a partial relapse after initially stable improvement because of inadequate immunosuppressive drug therapy, 4 were never stable but their improvement was successfully maintained with infrequent plasmaphereses, one relapsed completely when all therapy had to be withdrawn, and one had no response. The improvement attained was often striking. All 12 respirator patients became able to breathe independently and there were 3 complete remissions. Vital capacity increased by a mean of 65%, the arm extended time increased by 534%, the anticholinesterase medication requirement fell by 52%, and the electromyogram decremental response was reduced by 41%.

Serial anti-AChR antibody determinations were made in 14 of the 19 patients with stable improvement who took both prednisone and azathioprine during the phase of plasmapheresis and during the follow-up period (Figure 1). They received a mean of 11 plasmapheresis which lowered their anti-AChR titers by a mean of 71% at the end of plasmaphersis (2 to 3 months). At follow-up, 5 and 9 months after the onset of plasmapheresis, the mean anti-AChR antibody titers were still lower than the initial titer by 68 and 69%, respectively.

The results with our plasmapheresis regimen thus differ in three important respects from the results with intensive, short-term plasmapheresis in MG: (*a*) The majority of our patients attained stable clinical improvement; (*b*) there was a profound drop in anti-AChR antibody titers; and (*c*) anti-AChR antibody titers did not rise upon termination of plasmapheresis in stable patients. Over 1100 plasmaphereses have now been carried out in our unit without encountering any irreversible complications. At the present time plasmapheresis seems to offer a powerful and safe new therapeutic approach to patients who have not responded adequately to other treatment methods. A step-by-step summary of our present management of

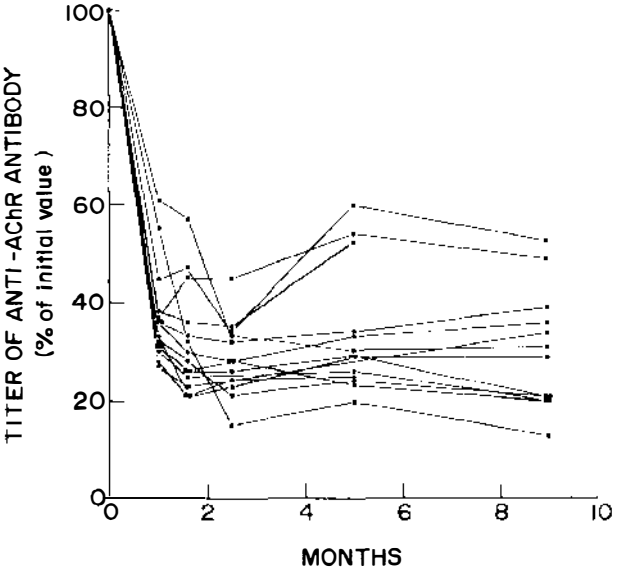

Figure 1 Serial determinations of antibody to AChR in patients treated by combined plasmapheresis and immunosuppressive drug therapy. Concentration of antibody to AChR in serum expressed as a percentage of the initial value is plotted as a function of time in 14 myasthenia gravis patients attaining stable improvement by means of plasmapheresis in combination with prednisone and azathioprine therapy.

MG includes: (*a*) anticholinesterase medications, (*b*) early thymectomy where indicated, (*c*) prednisone in patients not responding well to steps *a* and *b*, and (*d*) cytotoxic immunosuppressive drugs in combination with plasmapheresis in patients not responding adequately to steps *a, b,* or *c.* Cytotoxic immunosuppressives can be used alone in step *d*; however, for a given amount of risk to the patient from side effects, they are certainly more rapidly effective and powerful when used in conjunction with plasmapheresis.

How does reducing the concentration of anti-AChR by immunosuppressive drugs alone or in combination with plasmapheresis result in increased muscle strength? This has not been directly studied in patients, but one can extrapolate from our concepts of the pathological mechanisms in rats. Good clinical responses have been associated with sustained decreases of serum anti-AChR titers to 30% of the initial titer (76, 172, 173). What might this mean in terms of autoantibody/AChR? For example, take a 70 kg MG patient with the "average" concentration of anti-AChR in plasma [i.e. able to bind 5×10^{-8} mol ^{125}I-αbungarotoxin binding sites of AChR per liter (71)], and an "average" decrease in AChR content in skeletal muscle to

36% of the initial concentration (42) [from a normal level of 1×10^{-12} mol ^{125}I-αbungarotoxin binding sites/gm (42)], assume 40% of the body weight is muscle, and assume that the extracellular fluid compartment in which IgG is fully equilibrated is 7.8 1. Further, assume that when antibody titer is measured in AChR excess each antibody molecule binds two AChR molecules, each of which binds two ^{125}I-αbungarotoxin molecules (11). Then the ratio of anti-AChR antibody molecules to AChR molecules in the patient should be of the order 19:1. In this case, 70% reduction in anti-AChR titer as a result of therapy would reduce the ratio of antibodies to AChR is 6:1. None of the therapeutic approaches to MG eliminate anti-AChR completely; often anti-AChR concentration is higher than shown in this example, and percentage decrease in titer after therapy is often lower (171). Thus antibody must usually exist in substantial excess over AChR, and effective therapy presumably alters this ratio sufficiently to alter the dynamic equilibrium between AChR and membrane synthesis and antibody-induced AChR and membrane destruction in favor of greater equilibrium amounts of AChR and better-structured postsynaptic membrane. After a sustained decrease in serum anti-AChR content one would expect to observe greater AChR content in muscle, fewer of the AChR bound by antibodies, less complement bound to the membrane, and better-structured postsynaptic membrane. All these changes would be expected to increase the safety factor for neuromuscular transmission toward the normal range. Correlation between changes in anti-AChR titer in serum and changes in electromyogram decrement after plasmapheresis has been made in some patients (104). More sophisticated techniques for examining the pathology at the endplate are available. Measurements of AChR content (42, 62), antibody-bound AChR (42), postsynaptic membrane structure (64), bound complement (66), acetylcholine sensitivity (58), and endplate potentials (57) have been made in MG patients. However, no systematic study of changes in these parameters with therapy has been made.

CONCLUSION

Significant progress has been made toward understanding and controlling MG, in part through studies of EAMG. The primary cause of the muscle weakness which characterizes MG is loss of AChR, which reduces the acetylcholine sensitivity of the postsynaptic membrane in skeletal muscle. An autoimmune response to AChR mediated by antibodies rather than phagocytic cells is primarily responsible for loss of AChR. The loss of AChR is real rather than only apparent, as would be caused by competitive or noncompetitive inhibition of AChR by antibody. Loss of AChR occurs by accelerating the normal rate of AChR degradation (antigenic modula-

tion) and by complement-mediated focal lysis of the postsynaptic membrane. In addition to AChR loss, disruption of postsynaptic membrane structure and small direct effects of bound antibody on AChR probably further compromise neuromuscular transmission. Detection of antibody to human AChR in serum is a sensitive and objective method for diagnosis and monitoring response to therapy which can be used in addition to clinical evaluation. In MG, the safety factor for neuromuscular transmission is decreased. Treatment with inhibitors of acetylcholinesterase compensates in part for loss of AChR by increasing the concentration and duration of acetylcholine in the synaptic cleft, and is adequate symptomatic therapy in patients with mild disease. Most patients also require therapy directed at reducing the autoimmune response to AChR to permit a partial increase in AChR content and partial restructuring of the postsynaptic membrane. Effective therapy can increase the safety factor for transmission to the point that in some patients nearly normal function can be restored. Therapies in use for several years, thymectomy, corticosteroids, and cytotoxic drugs, have immunosuppressive effects. More recently, plasmapheresis in combination with immunosuppressive drugs has been used in severe cases to more specifically reduce the antibodies to AChR.

The cause (or causes) of the autoimmune response to AChR in MG is not known. Neither is a means for curing MG by specific immunosuppression known. The current studies of MG and EAMG are providing both data and techniques which may permit progress toward the solutions of these problems. Hopefully, these studies will also assist in the understanding of other autoimmune antireceptor diseases which are known (174–176) or suspected (177), as well as others yet to be recognized.

MG has proven interesting out of proportion to its incidence because it has involved fundamental processes and permitted a vital interaction between basic research into AChR structure, function, and metabolism, and medical research. This interaction is producing practical medical benefits.

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