Immune regulation of experimental myasthenia

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SUMMARY Experimental autoimmune myasthenia gravis (EAMG) is an appropriate model for studying the molecular origin, immunological mechanism and regulation of myasthenia gravis. Several approaches are being utilised for the regulation of the immune response to AChR and for immunosuppression of EAMG: Corticosteroids and azathioprine can suppress EAMG concomitantly with suppression of immune responses to AChR. High dose cyclophosphamide treatment in mice facilitates the onset of EAMG and results in a selective suppression of the humoral response to AChR whereas the cellular response is enhanced. Specific immunosuppression of EAMG is achieved by using a nonmyasthenic, denatured AChR preparation which cross reacts with the intact receptor. Various degradations and modifications of AChR are being performed in order to identify the smallest molecular entity responsible for the myasthenic activity of AChR. Studies on specific monoclonal antibodies, anti-idiotypes, and on the effect of measles virus on EAMG are being described and their possible significance in regulating myasthenia are being discussed.

Two decades have passed since it was first suggested that "myasthenia gravis is an 'auto-immune' response of muscle in which an antibody to end plate protein may be formed".¹ This hypothesis, which was originally based on attempts to explain clinical and experimental phenomena observed in myasthenia gravis (MG), has undoubtedly proved to be correct. Today, 20 years after the very first seeds of the autoimmune hypothesis were sown,^{1,2} it is well established that MG is an autoimmune disease in which acetylcholine receptor (AChR) is a major autoantigen.

A large part of the recent advances in our understanding of the nature and origin of MG is due to the ability to induce experimental myasthenia by immunisation of animals with purified AChR. This experimental disease which was designated experimental autoimmune myasthenia gravis (EAMG) and which was first observed almost accidentally in 1973, has become an extensively studied model system. We have accumulated considerable knowledge concerning both the molecular structure of AChR and the nature of the autoimmune response which it produces.

The availability of an experimental model disease for MG induced by a well defined antigen (AChR), provides a valuable tool for attempting regulation and suppression of the disease. Two general approaches for suppression of EAMG have been used in our laboratory: *non specific* and *specific* immunosuppressive treatment. While in the first approach we applied non-specific drugs which affect the whole immune system, in the latter we are aiming at specific suppression of that immune response to AChR which is responsible for its myasthenic activity. In this paper we describe experiments from our laboratory attempting regulation of the immune response to AChR and suppression of EAMG.

REGULATION OF EXPERIMENTAL MYASTHENIA BY NON-SPECIFIC IMMUNOSUPPRESSIVE DRUGS

Corticosteroids and azathioprine: Corticosteroids and the antimetabolite azathioprine were empirically found to be effective in the treatment of MG.³⁻⁶ We have shown previously that myasthenic patients have both cellular⁷ and humoral⁸ immunological sensitivity to AChR and that the cellular immune response to AChR is immunosuppressed following steroid therapy.⁹ We have

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used the same drugs in order to suppress or rather to prevent the onset of EAMG in rabbits and to follow the mechanism of suppression.¹⁰⁻¹² This system allows us to vary the course of the drug treatment and to analyse concomitantly immunological parameters affected by the treatment.

Rabbits injected with purified *Torpedo californica* AChR were treated with hydrocortisone either by early continuous administration of high doses of the drug or by administration of gradually increasing doses of hydrocortisone.¹¹ When hydrocortisone was administered in high doses from the beginning, some suppressive effect was observed although EAMG appeared earlier and sometimes in a more severe form than in the control animals. In the group of rabbits treated with gradually increasing doses of hydrocortisone, EAMG was completely suppressed. The schedule of steroids administration seems to be of great importance also in MG patients specially for minimising side effects; in spite of the overall beneficial effect of steroids in MG, increasing weakness usually occurs early in treatment, and can be avoided by a gradually increasing dosage schedule.

The effect of hydrocortisone in suppressing EAMG was paralleled by a diminished cellular sensitisation to AChR *in vitro*.¹¹ This is in agreement with our findings that in myasthenia gravis decreased cellular sensitisation to AChR correlated with clinical improvement during prednisone therapy.⁹

We have studied in more detail the immunosuppression of EAMG by prolonged treatment with azathioprine (Az) and have studied the correlation between clinical effects and several immunological parameters resulting from this immunosuppressive treatment.¹⁰⁻¹⁴ Rabbits were injected on day 0 with AChR (80 µg, in complete Freund's adjuvant, intradermally) and with Az (4 mg/kg, intramuscularly). Similar administrations of Az were given to the rabbits daily for 15 days and then every two to three days for an additional five months. In a control group the Az injections were replaced by saline injections. The Az treatment effectively suppressed the onset of EAMG in rabbits, for at least 12 months, even after discontinuing the drug treatment for 7 months (table 1).¹²⁻¹⁴ A second injection of AChR to such immunosuppressed rabbits, 12 months later, led to the onset of EAMG after a further six to ten days, as is the case after a secondary injection with AChR.

The suppressive effect of Az was accompanied by decreased cellular and humoral immunological reactivity against both the immunising *Torpedo*

Table 1 *Suppression of AChR-induced EAMG by azathioprine*

Treatment*	EAMG		Surviving after 12 months (%)
	Onset (days)	Clinical signs (%)	
Saline (control)	21-30	100 (8/8)†	0
Azathioprine (5 months)	100	10 (1/10)	80 (8/10)

*All rabbits were injected with 80µg *Torpedo californica* AChR on day 0.

†The numbers in parentheses represent the number of animals.

AChR and self AChR, with a significantly more pronounced effect on the response to self receptor.¹² The cellular sensitivity towards the *Torpedo* AChR as measured by the *in vitro* lymphocyte transformation technique, was suppressed by about 45% as a result of Az treatment, whereas the reactivity towards self receptor was essentially abolished. Az treatment was shown to be effective also on macrophage bound antibodies and to a lesser extent on lymphocyte bound antibodies. The effect of Az on the humoral response towards AChR was measured by analysing total circulating antibodies as well as cytophilic antibodies. The antibody titre against self AChR, representing an autoimmune response, was essentially abolished as was the case also for the cellular sensitivity. However, the titre against the foreign fish receptor was reduced only to a small extent.¹²

Az treatment resulted in qualitative changes in the antibody class, specificity, and affinity. The association between the immunosuppressive effect of Az on AChR-induced EAMG and the affinity of the antibodies was studied, utilising purified anti-AChR antibodies.¹³ Binding experiments were performed with constant amounts of purified antibodies reacted with increasing amounts of iodinated AChR. The data of these experiments were plotted using Sips and Scatchard equations.¹⁵ From the mathematical analysis obtained from these binding experiments it could be concluded that Az treatment leads to quantitative rather than qualitative differences. Thus it appears that the main immunosuppressive effect of Az is on the level of anti-AChR antibodies possessing high affinity values.¹³

The experiments with azathioprine allowed also the evaluation of the immunological memory following such an immunosuppressive treatment.¹⁴ As mentioned earlier a second injection of AChR to Az immunosuppressed rabbits, 12 months after

the first challenge led to the development of acute clinical signs of EAMG six to ten days later. This behaviour suggests that although EAMG was prevented by Az treatment, immunological memory was maintained. However, it is of interest to point out that no clinical myasthenic signs were observed in rabbits which were originally injected with AChR, immunosuppressed with Az and boosted 12 months later with denatured AChR preparation (RCM-AChR), which cross-reacts with AChR but does not induce EAMG.¹⁶ Thus, the anamnestic response to the myasthenic determinants was not awakened by this cross reactive, AChR derivative.

In addition to the clinical manifestation of the secondary antigenic challenge in the immunosuppressed rabbits the humoral immune response exhibited also an anamnestic pattern. The antibody titre in AChR-injected and Az treated rabbits decreased gradually during the first 12 months to a level of about 1% (3×10^{-9} M, expressed as moles of AChR precipitated by litre of serum,¹⁴) of the titre obtained 30 days after the primary injection (2×10^{-7} M). Following the booster AChR injection, there was a marked and fast increase of the antibody titre, to about 10^{-6} M. It should be noted that rabbits that were given a booster injection of RCM-AChR exhibit also an increase in anti-AChR antibody titre (to 10^{-7} M), although this increase was lower than that obtained in AChR boosted rabbits. The fast kinetic and extent of anti-AChR antibody in the boosted animals is similar to or even higher than a secondary response obtained in rabbits following a booster injection of AChR without any immunosuppressive treatment. The affinity of the antibodies elicited in the suppressed rabbits by the second immunisation, was at least as high or higher than that of rabbits immunised twice with AChR. The specificity of antibodies elicited in the Az immunosuppressed rabbits following the second antigenic stimulation is also identical to that of antibodies of non treated rabbits.

The utilisation of non-specific immunosuppressive drugs in an experimental animal system enables us to elucidate various aspects in their mechanism of action. However, there are no previous reports available on how immunological memory is affected in autoimmune diseases following immunosuppressive treatment. Our data¹⁴ demonstrate the existence of immunological memory following a prolonged Az treatment of AChR-injected rabbits. It seems that such rabbits still have the memory cells that could differentiate into effector cells (killer cells) and antibody producing

cells, leading to a concomitant appearance of the disease in a very short period of time. These particular memory cells are specific ones, and cannot be activated by the cross-reactive antigen RCM-AChR.

In spite of its immunosuppressive activity, it appears that even prolonged Az treatment does not affect the antigen sensitive cells that carry memory. This fact should be very carefully considered when immunosuppressive drugs are used in the treatment of autoimmune diseases since a very small challenge may recall an enhanced appearance of the disease even in patients who have undergone immunosuppressive treatment.

In conclusion, the immunosuppressive treatment of EAMG provides a valuable tool for elucidating many aspects of the mechanism of action of non specific immunosuppressive drugs in therapy of autoimmune diseases in general, and, in particular, for establishing the optimal conditions for such a therapy in MG.

Cyclophosphamide: Cyclophosphamide (CYP) is an immunosuppressive drug^{17, 18} known to have a selective effect on distinct cell populations involved in the immune response. Its effect is highly dependent on the dose and timing of administration with respect to the antigen injection. Given at high doses (100–300 mg/kg) CYP inactivates both B cells and suppressor T cells,¹⁷ and enhances some forms of delayed type hypersensitivity.^{19, 20} CYP was shown to be a potent suppressor of antibody response in the mouse²¹ due to its effect on B-cells. There are reports in the literature that CYP is also involved in the induction of endogenous viruses²²; Tindall *et al* have observed increased titres of cytomegalovirus antibodies in MG patients given CYP or Az, and appearance of virus in the urine.²³ It thus seemed of interest to study the effect of CYP on regulating the immune response to AChR and on the induction and course of EAMG in mice.

Different mouse strains possessing different H-2 haplotypes or on different genetic background such as C57BL/6J, BALB/c, SWR and ASW, were injected subcutaneously (between the ears) with CYP in saline (150–200 mg/kg). Forty hours later those mice were immunised intradermally with AChR (10 µg/mouse) in complete Freund's adjuvant (CFA). C57BL/6J mice were found to be the most sensitive to the CYP pretreatment and in many cases showed clinical signs of EAMG 6–8 weeks following the single immunisation with AChR. On the other hand such CYP pretreatment had no effect in mouse strains which were reported as not susceptible to the disease.²⁴

The humoral and cellular response of the CYP-treated and AChR-injected C57BL/6J mice, as well as of the control mice injected with AChR, without CYP pretreatment, was studied on various days following AChR administration. Humoral immune response was determined by micropassive haemagglutination test²⁴ and cell mediated immunity was determined by the ear test.²⁵ For this test the tested mice were injected subcutaneously with AChR (8 μ g) in the right ear and with diluting buffer in the left ear. Ten hours later FUDR (0.1 μ mole) followed by ¹²⁵I-UdR (2 μ Ci, given 30 minutes after FUDR) were administered intraperitoneally.²⁵ The ears were cut 26 hours after the AChR sensitisation and the radioactivity was counted. The results were expressed as the ratio of radioactivity of the right (experimental) to the left (control) ear (stimulation index—SI). As can be seen in fig 1 CYP treatment resulted in suppression of humoral response and enhancement of cellular response.

Fig 1 Kinetics of delayed type hypersensitivity and humoral response in C57BL/6J mice. Open bars and empty circles represent SI and log₂ haemagglutination titres, respectively, in C57BL/6J mice treated with CYP 48 hrs before AChR injection. Dark bars and dark circles represent SI and log₂ haemagglutination titres, respectively, in C57BL/6J mice injected with AChR and not treated with CYP.

It is thus suggested that the induction of unresponsiveness at the humoral level and the increase in the cellular reactivity associated with CYP treatment may have facilitated the onset of EAMG in C57BL/6J mice following one injection of AChR. However, it should be pointed out that whereas the effect on humoral and cellular activity by CYP was observed in all experiments, its effect on the onset of EAMG was not always reproducible. It is still not clear what is the mode of action of CYP that makes the animal more prone to EAMG but nevertheless it may be helpful in

the elucidation of the immunological mechanism involved in this disease.

MODIFICATION OF THE AChR MOLECULE AND SPECIFIC IMMUNOSUPPRESSION OF EAMG

Although immunosuppressive drugs seem to be useful in the treatment of both the human and experimental diseases, there are disadvantages in using such agents which may suppress the whole immune system. The ideal treatment of choice, if possible, should be a *specific* one, namely, a drug which will affect selectively the immunological reactivity which leads to the neuromuscular disorder, leaving the overall immune response intact. Having a well defined antigen (AChR) which induces EAMG and which is the autoantigen in MG, one may try by molecular modifications and derivations, and by immunological analysis of the receptor molecule to isolate and characterise small fragments of the molecule which are responsible for the specific cholinergic or the pathologic myasthenic activity of AChR, or both. Moreover, by such an analysis one may design and achieve derivatives of AChR with a potential to regulate *specifically* the immune response to AChR and to immunosuppress EAMG.

Studies with denatured AChR: We have shown previously that a denatured AChR preparation which by itself does not induce EAMG has a therapeutic potential on this disease.^{10 16 26} Denaturation of AChR was achieved by complete reduction and carboxymethylation of the receptor in 6M guanidine hydrochloride and the obtained derivative was designated reduced-carboxymethylated AChR or RCM-AChR. RCM-AChR is devoid of the pharmacologic activity of the intact AChR and it does not bind ¹²⁵I- α -bungarotoxin. It also lost the myasthenic activity of AChR as rabbits immunised repeatedly with RCM-AChR did not develop any clinical signs of EAMG. However, such rabbits developed high titres of antibodies which cross-react with AChR. Differences between the antigenic specificities of AChR and denatured AChR were observed by both immunodiffusion and radioimmunoassay (fig 2). AChR and RCM-AChR bind in an identical manner to anti-RCM-AChR whereas there is only a partial cross reactivity between anti-AChR and RCM-AChR, suggesting the presence in this antiserum of antibodies against some antigenic determinants which do not exist on the denatured receptor. Thus, the major difference between AChR and RCM-AChR leading to their different pathogenicity, resides in their different antigenic specificity. Some antigenic determinants in the AChR molecule were abolished

Fig 2 Specificity of anti-AChR and anti-RCM-AChR antibodies. (a) Immunodiffusion of anti-RCM-AChR serum (well 1) and anti-AChR serum (well 4) with RCM-AChR (wells 2, 3 and 5) and AChR (wells 6 and 7). (From Bartfeld and Fuchs.²⁶) (b) Inhibition of the binding of ¹²⁵I-AChR to anti-AChR serum (left) and anti-RCM-AChR serum (right) by AChR (—) and RCM-AChR (---). (From Bartfeld and Fuchs.¹⁶)

by the denaturation procedure. However, no additional determinants which were not expressed in the intact molecule became immunopotent after denaturation.

The difference in specificity between anti-AChR and anti-RCM-AChR is reflected also by their effects on the binding of bungarotoxin to AChR: whereas anti-AChR antibodies block the *in vitro* binding of ¹²⁵I- α -bungarotoxin to AChR very effectively, anti-RCM-AChR antibodies block this binding only to a very limited extent.²⁶

The altered antigenic specificity of antibodies to RCM-AChR along with their altered effect in blocking toxin binding to AChR led us to propose

that the denaturation of AChR destroyed one or more antigenic determinants, important for the induction of EAMG, and which may be located close to the toxin-binding site.

The immunological, pharmacological and pathological properties of RCM-AChR made it an appropriate potential candidate for trying specific immunosuppression of EAMG. Indeed, RCM-AChR which by itself is devoid of any myasthenic activity was shown to have both preventive and therapeutic effects on the experimental neuromuscular block induced by the intact receptor.^{10 16} The onset of EAMG was either delayed or completely prevented in rabbits preimmunised with RCM-AChR. In addition to the protective potential of RCM-AChR, its therapeutic effect on myasthenic rabbits was observed as well. A single administration of RCM-AChR in complete Freund's adjuvant to myasthenic rabbits during the initial stages of clinical development of EAMG led to a suppression of EAMG in at least 15 out of 30 myasthenic rabbits.^{10 16 27}

There seemed to be a correlation between the clinical conditions of the RCM-AChR treated rabbits and the antigenic specificity of their immune response. There were differences both in antibody titres and antigenic specificity between sera of rabbits in which EAMG was prevented or reversed by RCM-AChR and those of sick non-treated rabbits.^{16 27 28} The preventive as well as the therapeutic effect of RCM-AChR was accompanied by a change in the specificity of the humoral immune response, from a specificity of AChR-injected rabbits towards that of non-myasthenic RCM-AChR injected rabbits^{16 26-28} (fig 3). All the rabbits in which EAMG was eliminated following an injection of RCM-AChR exhibited a similar change of antibody specificity.

The cross-reactivity between AChR and RCM-AChR and the non-pathogenicity of the latter appear to be crucial in governing the immunosuppressive and therapeutic effects of RCM-AChR on EAMG. It is possible that the humoral responses which accompany the prevention, appearance or suppression of EAMG represent relative levels of two different antibody populations against AChR, one of which is specific to a myasthenic determinant in the AChR molecule and is involved in the disease, whereas the other one is directed to other antigenic determinants which are not involved in the disease. AChR can elicit antibodies to both types of determinants and it is the immune response against the myasthenic determinants which is responsible for induction of EAMG.

Fig 3 Therapy of EAMG by RCM-AChR. Correlation between the clinical symptoms and the antigenic specificity of the antibodies in a representative myasthenic rabbit in which the disease had been reversed by treatment with RCM-AChR. The inhibition of the binding of ^{125}I -AChR to the rabbit's serum by AChR (—) and RCM-AChR (---) was measured for each bleeding. The course of immunisations, bleedings and development of EAMG are described schematically at the top of the figure. (From Bartfeld and Fuchs.¹⁶)

By using an immunoabsorbent of RCM-AChR bound to Sepharose it is possible to fractionate these two antibody populations from antisera of AChR-injected rabbits.²⁹ The unadsorbed antibody fraction (effluent) is of antibodies which bind only to AChR and not to RCM-AChR. This antibody fraction is designated anti-native AChR (n-AChR) antibodies. It blocks the *in vitro* binding of toxin to AChR and probably contains antibodies against myasthenic determinants. The second antibody population (eluate) which adsorbs to RCM-AChR does not block toxin binding to AChR and it represents antibodies against non-structural determinants of AChR present also on the denatured receptor. Although the latter antibody fraction (eluate, or anti-denatured AChR (d-AChR) antibodies) may be present in myasthenic AChR-injected rabbits, it is probably not involved in the myasthenic activity and may even have a regulating effect on it.

The therapeutic potential of denatured AChR derivatives may have practical implications if similar approaches can be worked out also for AChR from other species or whether denatured *Torpedo* AChR can exert a therapeutic effect on myasthenia induced by AChR from other sources. *Trypsinated AChR*: Enzymic degradations of

AChR provide a useful approach for attempting the isolation and analysis of fragments of the receptor molecule which retain the specific cholinergic binding site or the pathologic myasthenic activity or both. Isolation and characterisation of the minimal structural entity which governs the myasthenic activity of AChR may provide a powerful tool towards designing specific drugs for myasthenia.

To this end we have treated AChR with trypsin and have studied the biological activity of the resulting derivative.³⁰ Tryptic digestion of AChR from *Torpedo californica* did not change the pharmacological specificity and the pathological myasthenic activity of the receptor molecule. The product obtained after tryptic digestion was re-purified by affinity chromatography on a *Naja naja siamensis* toxin-Sepharose resin and was designated T-AChR. T-AChR has a sedimentation coefficient of 8.0 S and in SDS acrylamide gel electrophoresis shows one major band with a molecular weight of about 27 000. Immunological analysis reveals that T-AChR bind to anti-AChR antibodies directed against conformational determinants of AChR (anti-n-AChR) and binds very weakly to antibodies directed against non-structured determinants (anti-d-AChR)^{29 30} (fig 4). This

is in contrast to the denatured receptor (RCM-AChR) which binds only to anti-d-AChR antibodies³⁰ (fig 4). These results suggest that the exposed unfolded regions of AChR were digested by the trypsin, retaining only the folded regions which are resistant to proteolytic activity. The cholinergic binding sites as well as the myasthenic sites of AChR seem to reside within this structured region. Immunisation of rabbits with T-AChR (one or two injections of 80 µg protein in complete Freund's adjuvant) results in EAMG symptoms identical to those obtained following immunisation with AChR.

Fig 4 Antigenic specificity of T-AChR. Inhibition of the binding of ¹²⁵I-AChR to anti-n-AChR antibodies (a) and to anti-d-AChR antibodies (b) by AChR (—), T-AChR (---) and RCM-AChR (···).

T-AChR represents an active receptor molecule with a lower structural complexity than that of the intact detergent purified receptor. It seems that the polypeptide chains in T-AChR result from the 40 000 polypeptide of the intact receptor, which is known to contain the cholinergic site. If this is the case it may be concluded that the myasthenic activity of AChR resides also within the 40 000 subunit of the receptor; this simplifies further studies for the elucidation of the molecular origin of the myasthenic activity of AChR and for designing specific drugs for myasthenia.

Other modifications: Additional manipulations of AChR are achieved by chemical modifications of specific functional groups in the molecule. Polyallanylation of free amino groups in AChR by reacting them with N-carboxy-DL-alanine anhydride resulted in a derivative designated poly-DL-alanyl AChR (PA-AChR).^{31 32} This chemical modification did not affect the physiologic activity

of the receptor as measured by toxin binding capacity but it abolished its myasthenic activity. Immunological studies demonstrated that polyallanylation resulted in a marked change in the immunogenicity but not in the antigenic specificity of AChR. Antibodies from rabbits immunised with PA-AChR were directed mainly to the polyallanine determinants and bound AChR only to a very limited extent. On the other hand the antigenic reactivity of PA-AChR towards anti-AChR antibodies was as efficient as that of AChR. Such immunological specificity may render PA-AChR an effective agent for immunosuppression of EAMG by antigenic competition or, alternatively, by neutralising the immune response to AChR.

Nitrated AChR obtained by reacting AChR with tetranitromethane was demonstrated to be devoid of both myasthenic and pharmacologic activity (Mochly-Rosen and Fuchs, in preparation). This chemical modification is known to affect specifically tyrosine residues in proteins^{33 34} and it is thus suggested that at least some tyrosine residues in AChR are crucial for its biological activity.

Additional chemical modifications of AChR and its further enzymic or chemical degradation will contribute to the detailed structure and function analysis of this molecule. Such an approach may lead to the elucidation of a small therapeutic fragment and its synthesis can be then attempted. If such a fragment would be common to various nicotinic receptors its therapeutic potential might be broader.

Monoclonal antibodies

The involvement of anti-AChR antibodies and of specific cell mediated immunity in the pathogenesis of MG and EAMG has been demonstrated on clinical and experimental grounds. Passive transfer of immunoglobulins from myasthenic patients³⁵ and from animals with EAMG³⁶ resulted in myasthenia gravis-like symptoms. AChR is a complexed multideterminant immunogen which raises a heterogeneous immune response and it is still not known which and whether certain specificity (ies) is (are) responsible for the myasthenic activity of the molecule. In order to analyse the structure of AChR and for identifying the molecular entity which governs its myasthenic activity, large quantities of antibodies with defined specificity are required. Such antibodies provide a useful tool for studying the role of antibodies in myasthenia and their mechanism of action. To this end we have used the method of lymphocyte hybridisation developed by Köhler and Milstein³⁷ and have prepared hybridomas by hybridisation of mouse

anti-AChR antibody producing cells with a non-secreting myeloma line. Such hybridomas secrete monoclonal anti-AChR antibodies with restricted specificities towards defined determinants of AChR.³⁸

Our findings suggest that some of the hybridomas secrete antibodies which recognise selectively conformational antigenic determinants of AChR, which are present in the trypsinated receptor³⁰ and are absent in the denatured receptor.²⁶ The myasthenic activity of the receptor molecule resides within this group of determinants. Thus it may be expected that antibodies directed against these specificities may have pathological effect associated with myasthenic activity. On the other hand, other hybridomas secrete antibodies which recognise selectively denatured AChR, which expresses the non-structural antigenic determinants of the receptor molecule. Such antibodies are probably not associated with any myasthenic activity and may even have a perverted or therapeutic activity.

We have recently developed one hybridoma which secretes antibodies directed against the cholinergic binding site of AChR. The binding of these monoclonal antibodies to AChR is inhibited by bungarotoxin and by several cholinergic agents. Although this antibody specificity is not detected in immune anti-AChR sera, it may be present there in minute quantities and may even play a crucial role in the pathogenesis of myasthenia. The ability to select preferentially for one specificity, even a minor one, is one of the advantages offered by the lymphocyte hybridisation method.

Thus, monospecific antibodies appear to be a useful tool for structural and functional analysis of AChR; they may help to define the sites involved in the various biological activities related to AChR. Moreover, they may enable the elucidation of the role of antibodies of defined specificity, in regulating the immune response to AChR and the disease resulting from it.

Anti-idiotypes

Anti-idiotypes may have a regulatory role in auto-immune diseases or other disorders of an immunological nature. The application of anti-idiotypic antibodies furnishes a possible approach for specific regulation of the immune response to AChR and of myasthenia. Anti-idiotypic serum specific to anti-AChR idiotypes was prepared in C57BL/6J mice by repeated injections with purified C57BL/6J anti-AChR antibodies or with syngeneic spleen cells educated with AChR.³⁹ The anti-idiotypic serum reacted specifically with anti-

AChR antibodies of several mouse strains and of other species. The anti-idiotypic serum was also able to inhibit the binding of ¹²⁵I-AChR to mouse anti-AChR antibodies, suggesting that idiotype determinant(s) against which the serum is directed are associated with the antigen combining site.³⁹ The broad cross-reactivity among anti-AChR idiotype determinants of different mouse strains as well as those of different species suggest that similar idiotype determinants exist in the anti-AChR antibodies tested.

The approach of anti-idiotypes is hampered by the use of a heterogeneous population of anti-AChR antibodies for the elicitation of anti-idiotypic serum. Antibodies obtained from AChR-immunised animals contain a whole variety of different idiotypes, part of which may not be relevant for myasthenia. Now that monoclonal anti-AChR antibodies are available it may be advantageous to raise anti-idiotypic serum against monoclonal antibodies of characterised defined specificity.

Measles virus (possible viral involvement?)

The etiology of MG is still not clear; a viral infection has been proposed as one of the possible triggering factors.⁴⁰ Certain diseases of the central nervous system such as subacute sclerosing panencephalitis⁴¹ and possibly multiple sclerosis⁴² are associated with measles virus infection. In view of this and also since we faced some difficulties in obtaining reproducible EAMG with high incidence in mice, we have studied recently the effect of measles virus vaccine on the induction of EAMG in mice (Feingold, Tarab-Hazdai and Fuchs, in preparation). Our preliminary results indicate that injection of measles virus vaccine in combination with AChR facilitate the onset of EAMG in C57BL/6J mice. The results of three experiments are summarised in table 2. In all three experiments an early onset of EAMG was observed following one injection of AChR together with measles virus vaccine. The specificity of the disease was confirmed by the Tensilon test. With AChR alone at least two injections were required for the induction of EAMG.²⁴ The effect of the measles virus appeared to be restricted to mice susceptible to EAMG, since immunisation of SJL/J mice (a non-susceptible strain²⁴) with a combination of AChR and measles virus vaccine had no effect on the induction of the disease. Also, the stimulating effect of measles virus vaccine on C57BL/6J mice was not observed by another type of virus such as bacteriophage T₄.

The mechanism by which measles virus regulates

Table 2 Effect of measles virus vaccine on EAMG

Treatment	Day tested	Score of clinical signs
Exp. 1	AChR + measles on day 0; AChR on day 140	30 0
		40 ++
		45 +++
		50 +++
		145 +++
	AChR on day 0; AChR on day 140	180 +++
		30 0
		50 0
		145 ++
	Measles on day 0; AChR on day 140	180 ++
		30 0
		50 0
		145 0
		180 0
Exp. 2	AChR + measles on day 0; measles on day 75	15 +
		30 +++
		45 ++
		85 +++
		110 +++
	AChR on day 0; measles day 75	125 +++
		30 0
		45 0
		85 ++
		110 +
	Measles on day 0; Measles on day 75	125 ±
		30 0
		45 0
		30 ±
		110 0
Exp. 3	AChR + measles on day 0; AChR + measles on day 35	125 ±
		5 +
		30 ++
		40 +++
		50 +++
	AChR on day 0; AChR on day 35	30 0
		40 0
		60 0
	Measles on day 0; measles on day 35	30 0
		40 0
		60 0

C57BL/6J mice (7 mice in each group) were injected in the footpads in CFA with AChR (10 µg), measles virus vaccine (25 µl from a 1 ml solution of live attenuated virus preparation (Merck, Sharp and Dohm)) or a combination of both. The mice were observed daily for signs of muscular weakness and other visual symptoms of the disease which were graded according to the following score: 0=no weakness; ±=weak grasping and fatigability in part of the group; +=weak grasping and generalised weakness in the whole group; ++=ruffled fur; hunched posture, uncoordinated movements and sometimes paralysis of limbs; +++=loss of ability to grasp, tremulous, severe generalised weakness.

EAMG is still not worked out. Its effect may be exerted by an adjuvant effect or by cross reactivity with AChR. Preliminary experiments support the latter possibility, as it was demonstrated that a second immunisation of mice with measles virus, following a first challenge with AChR or AChR and measles virus, has a boosting effect on the anti-AChR antibody titre. Furthermore, in some experiments mice immunised with measles virus alone had a low but significant anti-AChR anti-

body titre. Additional data should be accumulated before a conclusion can be drawn concerning the mechanism of the measles virus effect, and whether it can somehow explain its involvement in the aetiology of the disease.

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