We examined sera from 113 patients with myasthenia gravis (MG). Most of the patients with ocular MG without thymoma and 15% of the patients with generalized MG had immunoprecipitation (IP) titers of anti-acetylcholine receptor (anti-AChR) antibodies within the normal range for healthy subjects. We developed a highly sensitive radioassay using *Staphylococcus aureus* cells, and re-examined the 86 serum samples that had negative titers by IP. Using the radioassay, we detected anti-AChR antibodies in 27 (31%) of these myasthenic sera, of which 19 were from ocular MG patients without thymoma. By combining the standard IP assay and our new radioassay, we increased to 50% the overall percent positivity of detecting nonblocking-type antibodies in ocular MG patients without thymoma. We detected no anti-AChR antibodies in nearly all patients with various immunological and neurological diseases other than MG, and in all the healthy controls. The data for these sera indicate that in some cases the standard IP assay gives false-negative reactions. Thus, use of the more sensitive radioassay is preferable for accuracy.

Additional Keyphrases: *Staphylococcus aureus* cells • immunoprecipitation • α-bungarotoxin

The muscular weakness and fatigability seen in myasthenia gravis (MG) result from an antibody-mediated immune response to acetylcholine receptor (AChR). Among anti-AChR antibodies, non-blocking-type antibodies measured by the standard immunoprecipitation (IP) assay were detected in 85–90% of myasthenic patients (1–3), and shown to have a key role in producing the basic defects in MG. Therefore, detection of circulating anti-AChR antibodies is present the clinical standard for definitive diagnosis of MG. In a previous study (3), involving sera from a large number of Japanese patients with MG, we confirmed that patients with generalized MG had a high prevalence (85%) of anti-AChR antibodies. Particularly, the patients with thymoma showed a remarkably high frequency (98%) of non-blocking-type antibodies, in comparison with those without thymoma (80%). Of the patients with ocular MG, nearly all those with thymoma had positive titers for anti-AChR antibodies. In contrast, ocular MG patients without thymoma had rarely detectable antibody by IP assay (frequency, 8 of 57, 14%). Thus, most of the patients with ocular MG but without thymoma, and 15% of the patients with generalized MG, had titers of anti-AChR antibodies within the range for the healthy controls (mean + 2SD) determined by IP assay. We could not determine whether such MG patients had anti-AChR antibody titers less than the detection limit of the IP assay or whether they had different types of anti-AChR antibodies, which none of the present assays can measure.

To resolve this question, at least partly, we developed a highly sensitive radioassay, using *Staphylococcus aureus* cells, and re-examined the serum samples that had shown a negative titer by the IP assay.

Materials and Methods

Subjects. We examined 113 myasthenic sera obtained from 27 patients with detectable anti-AChR antibody and 86 patients with no detectable anti-AChR antibody by the standard IP assay. The IP-negative sera were from 50 patients with ocular-type MG, 31 patients with generalized MG without thymoma, three patients with ocular-type MG with thymoma, and two patients with generalized MG with thymoma. These 113 patients had typical clinical features and electromyographic evidence of this disease. The control population was 98 patients with other neurological diseases—eight with systemic lupus erythematosus; two with Sjögren's syndrome; 27 with rheumatoid arthritis, including 16 being treated with D-penicillamine; nine with polyomysitis; 10 with amyotrophic lateral sclerosis; 12 with spinal muscular atrophy; five with polynuropathy; two with Wilson's disease; 16 with primary biliary cirrhosis; and seven with thymoma without MG—and 20 healthy persons.

Preparation of radiolabeled AChR complex: Preparation of 125I-labeled α-bungarotoxin (α-BuTx) and the human AChR was as described previously (3). The specific activity of 125I-labeled α-BuTx used in this assay was about 1000 counts/min per femto-mole. The human AChR solution was incubated with a fourfold excess of 125I-labeled α-BuTx overnight at 4 °C, and the resulting 125I-labeled α-BuTx-AChR complex was fractionated from free 125I-labeled α-BuTx on a column of Sephacryl S-300 (Pharmacia Fine Chemicals, Uppsala, Sweden) equilibrated with 50 mmol/L Tris · HCl buffer (pH 7.4) containing 0.1 mol of NaCl and 2 mL of Triton X-100 per liter (buffer A).

IP assay: We performed the IP assay as previously described (3), with use of the crude AChR preparation from human skeletal muscles.

Radioassay: To develop this sensitive assay, we modified the standard IP assay by using *S. aureus* cells for trapping the IgG immune complex as a second antibody instead of performing the immunoprecipitation with a second antibody. Briefly, we pre-incubated 200 μL of a 100 g/L *S. aureus* cell suspension ("Pansorbin"); Calbiochem Corp., San Diego, CA) with 10 or 20 μL of test serum for 16 h at 4 °C, and then washed this twice by centrifuging at 3000 × g at 4 °C for 10 min with buffer A. We added 100 μL (200 fmol) of the 125I-labeled α-BuTx–AChR complex to the cell suspension and further incubated for 16 h at 4 °C. After washing the cells three times with isotonic saline and

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4 Nonstandard abbreviations: MG, myasthenia gravis; AChR, acetylcholine receptor; IP, immunoprecipitation; and α-BuTx, α-bungarotoxin.

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centrifuging, as above, we counted the radioactivity in the cell pellet by using a gamma counter. In this assay, values > 0.07 nmol/L (the mean for the normal controls plus 3 SD: 0.04 ± 0.03 nmol/L) were considered positive.

Results

The standard curve for anti-AChR antibody by this assay showed a linear relationship between amount of 125I-labeled α-BuTx capable of binding to the cells and the amount of the anti-AChR antibody in the range of 0.07–5 nmol/L. Therefore, this assay system may be applicable to serum samples with anti-AChR antibody titers less than the detection limit (< 0.6 nmol/L) of the standard IP assay. Figure 1 shows that this assay also detected all those sera that were positive by IP assay. The accuracy and specificity of this method were confirmed by the good correlation with results by the IP assay in these positive cases (r = 0.91).

We also re-examined 86 myasthenic sera for which titers of anti-AChR antibodies were within the normal range (< 0.6 nmol/L) by the IP assay (Figure 2). The radioassay detected anti-AChR antibodies in 19 of 50 subjects with ocular MG without thymoma, in five of 31 with generalized MG without thymoma, in two of three with ocular MG with thymoma, and in one of two with generalized MG with thymoma. In all, 31% (27 of 86) of the IP-negative myasthenic sera tested had positive titers by radioassay, although these titers were extremely low (0.07–0.6 nmol/L). Most of these 27 cases (19) were from ocular MG patients without thymoma. By using the combination of the standard IP assay and our modified radioassay, we increased the overall percent positivity of non-blocking-type antibodies in ocular MG with thymoma to 48%.

We also used the radioassay to screen various immunological and neurological diseases other than MG. Nearly all of these sera and all healthy control sera showed no titers of anti-AChR antibodies, although low antibody titers were found in two cases with thymoma but not MG (Figure 2).

Discussion

Non-blocking-type anti-AChR antibodies detected by the IP assay have been thought to be the major antibodies in MG sera because of their high frequency of detection.

However, this new assay detects the non-blocking-type antibodies against sites other than the ACh-binding site proper, similar to the standard IP assay. In this new assay, the detection limit was increased by about ninefold because larger serum volumes (20 vs. 0.5 μL) can be used and nonspecific binding of radioactivity in the pellet is avoided. S. aureus cells used to trap the IgG immune complex in this assay system do not bind IgG3 antibodies. However, in our IgG subclass analysis of anti-AChR antibodies, all Japanese MG patients we tested had high antibody activity almost exclusively in IgG1 subclass, with slight activity in IgG2 and IgG3 subclasses (4). Therefore, the failure to find IgG3 antibodies is not important and can be ignored.

Re-examination of IP-negative cases showed 31% of myasthenic sera to have positive titers, although the concentrations were extremely low (0.07–0.6 nmol/L). But the prevalence rate (50%) and titers of AChR antibodies in ocular MG were still remarkably lower than those detected in generalized MG when human gastrocnemius muscles were used as antigen. Others (5, 6) have found that the titers of anti-AChR antibody measured with human extracocular muscles were higher in ocular MG than were those measured with foot muscle. Compston et al. (7) reported that ocular patients have low anti-AChR, but also that these antibodies react equally well with AChR extracted from ocular or limb-muscle tissue, whereas sera from patients with generalized disease react better with AChR from limb muscle. When we measured anti-AChR antibodies by using bovine extracocular muscles as antigen, we could not find detectable antibodies in ocular MG patients (data not shown).

We detected extremely low titers of anti-AChR antibodies in two of the seven patients with thymoma but no MG. Similar observations have been reported for the standard IP assay (8–10). We have observed some patients who developed MG during treatment with D-penicillamine for rheumatoid arthritis. These patients had increased titers of anti-AChR antibodies, whereas after withdrawal of the drug, the titer of the antibodies decreased rapidly and any clinical sign of neuromuscular disorder rapidly disappeared. Using the radioassay, we examined the AChR antibodies in 10 patients with rheumatoid arthritis while they were receiving D-penicillamine treatment; these pa-

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Fig. 1. Correlation between titers of anti-AChR antibodies in sera from MG patients measured by an improved radioassay and by a standard IP assay

All these samples showed positive titers by IP

Fig. 2. Anti-AChR antibody concentrations measured by a radioassay involving S. aureus cells in samples that showed negative titers by IP assay

Upper limit of normal is shown by broken line
patients had neither anti-AChR antibodies by IP assay nor any clinical sign of MG. No detectable anti-AChR antibodies were found in these patients. Kyriatsoulis et al. (II) reported a high incidence of positivity in patients with primary biliary cirrhosis, but we could not detect any anti-AChR antibodies in these patients.

Our data indicate that this new assay is reliable in serum samples having relatively low or negative antibody titers by the IP assay and may have great potential for clinical use. However, we found that 10–15% of MG patients still have no detectable AChR antibodies: such cases will need re-evaluation with more specific and sensitive tests before any conclusions about them can be drawn.

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References

Effects of Recombinant Leukocyte Interferon on Ribonuclease Activities in Serum in Chronic Hepatitis B

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Alkaline ribonuclease (RNase; EC 3.1.27.5) activities and 2',5'-oligoadenylate synthetase (2-5 AS; no EC no. assigned) activities in serum were measured in nine patients with hepatitis B e antigen-positive chronic hepatitis B before, during, and after treatment with recombinant human interferon α-2b for four weeks at daily doses ranging from 3 to 10 mega-units. Alkaline RNase activities in serum significantly increased from 65.8±9.5 units/L (mean±SD) to 84.3±11.9 units/L after the first week of therapy (P<0.001). (One unit of RNase activity is defined as that increasing the absorbance at 260 nm by 1.0 in 1 min.) This increase persisted during and until two weeks after the end of the therapy, at which time the mean RNase activity in serum was still significantly increased (70.8±9.4 units/L, P<0.01). Before therapy, phosphocellulose chromatography of RNase showed five active peaks of enzyme activity, which were similar to that observed even when RNase activity increased immediately after therapy. There was a close correlation between RNase activities and the logarithm of 2-5 AS activities, measured simultaneously in each patient. We conclude that recombinant α-interferon therapy increases RNase activities in serum, associated with the increased 2-5 AS activities.

The antiviral action of interferon (IFN) has been demonstrated to be mediated by activation of endonuclease, which degrades viral messenger ribonucleic acid (RNA).1 In IFN-treated cells, IFN-induced 2',5'-oligoadenylate synthetase (2-5 AS) catalyzes the synthesis of 2',5'-oligoadenylate (2-5 A) from ATP in the presence of double-stranded RNA, and 2-5 A activates endonuclease (1–3). Because IFN may inhibit viral replication in this way, endonuclease may play an important role in the antiviral action of IFN. Increased concentrations of ribonuclease (RNase; EC 3.1.27.5) activity in human serum have been reported in cases of renal failure (4, 5), pancreatic cancer (6), multiple myeloma (7), leukemia (8), and other diseases (9). However, the effect of IFN therapy on RNase activity in serum has not been previously reported.

Recently, recombinant human leukocyte IFN has been used for treating chronic hepatitis B virus (HBV) infection.

1 Nonstandard abbreviations: 2-5 AS, 2',5'-oligoadenylate synthetase; IFN, interferon; 2-5 AS, 2',5'-oligoadenylate; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; HBeAg, hepatitis B surface antigen; ALT, alanine aminotransferase; AST, aspartate aminotransferase; and RNase, ribonuclease.

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