SHORT REPORT

Antibodies to ^{125}I -glutamic acid decarboxylase in patients with stiff man syndrome

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Abstract

Antibodies to glutamic acid decarboxylase (GAD) are found in about 40% of patients with stiff man syndrome. A new assay involving immunoprecipitation of ^{125}I -glutamic acid decarboxylase was used to measure anti-GAD antibodies in 18 patients with stiff man syndrome. Of the eight serum samples from patients with stiff man syndrome, that had previously been found positive by immunoprecipitation of ^{35}S -GAD, seven were strongly positive with ^{125}I -GAD and one gave an equivocal result. Other serum samples from patients with stiff man syndrome and from controls were negative except one from a patient who had a thymoma, acquired neuromyotonia, and myasthenia gravis. Nine of 35 serum samples referred for testing were positive; in two of these the serum titre was 20–50 times higher than that in the CSF. This assay should prove useful in the diagnosis, management, and investigation of stiff man syndrome.

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Stiff man syndrome is a rare neurological disorder in which muscle rigidity and cramps are thought to result from immune mediated inhibition of GABAergic neuron function. Some cases occur in association with insulin dependent diabetes mellitus and others are paraneoplastic, with breast tumours being most common. For a recent review see Pender.¹

Antibodies to glutamic acid decarboxylase (GAD) were first identified in stiff man syndrome associated with insulin dependent diabetes mellitus,^{2,3} and were subsequently shown to be present in some cases of stiff man syndrome without diabetes,⁴ and in many cases of diabetes without neurological disease.⁵ Anti-GAD antibodies are present in around 40% of patients with stiff man syndrome. They have not been identified in paraneoplastic stiff man syndrome, in which antibodies to amphiphysin

are typically present.^{4,6,7} The measurement of anti-GAD antibodies has previously relied on immunoprecipitation of ^{35}S -methionine labelled GAD produced in cell free lysates, or immunoblotting of recombinant GAD, or of rat or human brain homogenates. These methods are not easily available to routine laboratories and are impractical for quantitative studies. Here, we used an assay based on immunoprecipitation of ^{125}I -recombinant GAD which has been shown to detect anti-GAD antibodies in insulin dependent diabetes mellitus.⁸

Methods

We tested 22 samples (coded by IT and LMEG). Eight were from patients with stiff man syndrome who had previously been found positive for anti-GAD antibodies by immunoprecipitation of ^{35}S -methionine-recombinant GAD; 10 were from patients with stiff man syndrome previously found negative for anti-GAD antibodies, including four with cancer. Three were from healthy subjects and one was from a patient with chronic inflammatory demyelinating polyneuropathy (CIDP). As further controls, we tested 15 serum samples from patients with myasthenia gravis with thymoma, all of whom were positive for anti-acetylcholine receptor (anti-AChR) antibodies; 30 serum samples from patients with acquired neuromyotonia, 13 of whom had positive titres of antibodies to voltage gated potassium channels⁹; and 47 serum samples referred to us that had proved negative for both antibodies. Three control serum samples from healthy subjects were run with each assay. In addition, we have since tested 35 serum samples and two CSF samples referred by clinicians for anti-GAD antibody testing.

^{125}I -GAD was obtained from RSR Ltd (Cardiff, UK) and used according to the instructions with slight modifications. Serum (0.5–5 μl) was diluted 1:10 in 0.02 M phosphate pH 7.2/0.1% triton X-100 (PTX) buffer and added to 25–50 μl of ^{125}I -GAD (10 000–30 000 total cpm) for two hours at room temperature. Excess anti-IgG was added, the precipitates spun at 5000 rpm for two minutes, washed in PTX, and counted.

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Figure 1 Results of ^{125}I -GAD antibody assay in coded samples. Eight samples had previously been found positive (filled symbols) for anti-GAD antibody; these included five with stiff man syndrome and three with stiff man syndrome and insulin dependent diabetes mellitus. Of the 14 anti-GAD negative serum samples tested, 10 had stiff man syndrome, and four of these were associated with cancer (two breast, one colon, one Hodgkin's lymphoma); three serum samples from healthy controls and one from a patient with CIDP were included. The cut off (5.4%) was based on the mean (± 3 SDs) of these four and three additional serum samples from healthy controls. Results are expressed as a percentage of total ^{125}I -GAD precipitable by the standard positive control.

Results

We first tested 5 μl of each of the 22 coded samples. Seven of the test serum samples precipitated > 8500 cpm, and one other precipitated 1589 cpm. The three coded healthy control serum samples and the serum from a patient with CIDP precipitated between 285 and 757 cpm. Figure 1 gives the results expressed as % of total cpm. The mean for the

Figure 2 Titration of two stiff man syndrome serum samples and comparison with CSF. The titres were calculated from the linear part of the titration curve, and CSF values expressed as a percentage of serum sample values (results shown in the figures).

four coded and three additional healthy control serum samples was 1.8 (SD 1.2)%. Anti- ^{125}I -GAD antibodies were clearly positive in seven patients who had previously been found positive, equivocal (5.5%) in one other, and negative in all those previously found negative. The serum samples that precipitated $> 90\%$ of the available ^{125}I -GAD with 5 μl also precipitated $> 90\%$ with 1 μl indicating high titres of antibody (data not shown).

From samples referred for anti-GAD antibody assay, nine serum samples and two CSF samples were clearly positive. Figure 2 shows titrations of two serum samples with their respective CSFs. The anti-GAD antibody concentrations in CSF were substantially raised, but only represented 1.9 and 5.7% of the serum concentrations. Two control CSFs tested were negative.

We also assayed 45 serum samples from patients with other neurological diseases. Of 15 patients with myasthenia gravis associated with thymoma and 30 with acquired neuromyotonia, one had clearly raised anti-GAD antibodies, precipitating > 6000 cpm/5 μl serum. This patient presented with neuromyotonia, with positive titres of antibodies to voltage gated potassium channels, but she also had a thymoma and antibodies to acetylcholine receptor. She had no history of diabetes. The remaining serum samples, and a further 47 serum samples referred to us for diagnostic anti-AChR or antivoltage gated potassium channel antibodies, were all clearly negative, precipitating less than 600 cpm/5 μl (not shown).

Discussion

Stiff man syndrome is a rare neuromuscular disorder of presumed autoimmune origin in which specific autoantibodies have been detected, either to GAD, amphiphysin, or other undefined neuronal antigens.¹ Although the pathogenic role of humoral immunity has not been clearly shown, several reports have indicated a beneficial effect of plasmapheresis or immunosuppressive therapy, or both in some, but not all, cases.¹⁰⁻¹² The ready availability of an assay for measuring anti-GAD antibodies during and after such treatments may help to clarify the role of antibodies in the disease process.

Because anti-GAD antibodies and oligoclonal bands are found in the CSF of some patients with stiff man syndrome,^{2,3} it is possible that intrathecal synthesis of anti-GAD antibodies occurs. However, using this assay, the concentrations of anti-GAD antibodies in CSF are considerably lower than those in the patients' serum samples, making it unlikely that a substantial amount of the serum antibody originates intrathecally.

Acquired neuromyotonia is another disease that has recently been shown to be immunologically mediated with antibodies to voltage gated potassium channels.^{9,13,14} Paraneoplastic forms of both neuromyotonia and stiff man syndrome have been reported.^{1,15} It is intriguing that one patient with neuromyotonia asso-

ciated with thymoma studied here also had antibodies to AChR and GAD.

Stiff man syndrome is not a common condition, but of 35 serum samples sent by clinical neurologists, nine were clearly positive, confirming the diagnosis of stiff man syndrome. Further studies will be needed to establish the incidence of anti-¹²⁵I-GAD antibodies in stiff man syndrome, and to compare the titres with those in insulin dependent diabetes mellitus. The availability of this simple and cheap immunoprecipitation assay should prove useful in the diagnosis, management, and further investigation of these conditions.

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