grated during embryogenesis and remained in the basal layer of the endometrium. One of the AFP producing endometrial tumours was a mixed mesodermal type and it was suggested that malignant transformation of müllerian epithelial and stromal cells had occurred independently following differentiation of primitive mesenchymal cells into epithelial and stromal components.⁵ Two other poorly differentiated AFP producing endometrial tumours were papillary adenocarcinomas.^{6,7} The latter three AFP producing tumours showed no evidence of yolk sac differentiation. Matsukuma and Tsukamoto⁶ suggested that the tumour they studied had originated from abnormally differentiated endometrial columnar cells which produced AFP. Kubo *et al*⁷ concluded that AFP producing tumours may arise from müllerian duct elements. The present case is an example of hepatoid adenocarcinoma of the uterus, because of the close resemblance of the neoplastic cells to hepatoid cells and because of the production of bile canaliculi-like structures, which reacted with the polyclonal but not the monoclonal antibody directed against CEA.⁸ Hepatoid adenocarcinomas have been reported in the genitourinary system, probably as a result of metaplastic or transdifferentiation processes.⁹

AFP in the present case was diffusely localised to the cytoplasm. Reverse transcription polymerase chain reaction (RT-PCR) analysis of cDNA generated from the RNA present in the tumour cells using primers specific for AFP demonstrated the expression of AFP mRNA. AFP in the serum of our patient was purified by immunoaffinity chromatography and characterised. The results obtained on SDS polyacrylamide gel electrophoresis both before and after treatment with glycopeptidase F were indistinguishable from those of hepatoma derived

AFP. This indicated that the AFP derived from the tumour was also composed of a single polypeptide chain with a molecular weight of 67 000 and one N-linked sugar chain (molecular weight 3000). The 10 amino acid N-terminal sequence of the AFP derived from the endometrial adenocarcinoma was identical with that of the hepatoma derived form. AFP isoforms resulting from minor structural differences of carbohydrate moieties have been demonstrated by lectin affinity electrophoresis.¹⁰ Such analysis indicated that the isoform profile of this AFP is unique and distinct from the AFP derived from hepatomas, yolk sac tumours or cord serum. The detailed results of the biochemical characterisation of the AFP isolated from the present case will be reported elsewhere.

- 1 Pileri S, Martinelli G, Serra L, Bazzocchi F. Endodermal sinus tumors arising in the endometrium. *Obstet Gynecol* 1980;56:391-6.
- 2 Ohta M, Sakakibara K, Mizuno K, Kano T, Matsuzawa K, Tomoda Y, *et al*. Successful treatment of primary endodermal sinus tumor of the endometrium. *Gynecol Oncol* 1988;31:357-64.
- 3 Clement P, Young R, Scully R. Extraovarian pelvic yolk sac tumors. *Cancer* 1988;62:620-6.
- 4 Joseph M, Fellows F, Hearn S. Primary endodermal sinus tumor of the endometrium. A clinicopathologic, immunocytochemical, and ultrastructural study. *Cancer* 1990;65:297-302.
- 5 Kawagoe K. A case of mixed mesodermal tumor of the uterus with alpha-fetoprotein production. *Jpn J Clin Oncol* 1985;15:577-83.
- 6 Matsukuma K, Tsukamoto N. Alpha-fetoprotein producing endometrial adenocarcinoma: Report of a case. *Gynecol Oncol* 1988;29:370-7.
- 7 Kubo K, Lee G-H, Yamauchi K, Kitagawa T. Alpha-fetoprotein-producing papillary adenocarcinoma originating from a uterine body: A case report. *Acta Pathol Jpn* 1991;41:399-403.
- 8 Ma CK, Zarbo RJ, Frierson HF, Lee MW. Comparative immunohistochemical study of primary and metastatic carcinomas of the liver. *Am J Clin Pathol* 1993;99:551-7.
- 9 Sinard J, Macleay L, Melamed J. Hepatoid adenocarcinoma in the urinary bladder. *Cancer* 1994;73:1919-25.
- 10 Yamashita K, Taketa K, Nishi S, Fukushima K, Ohkura T. Sugar chains of human cord serum α -fetoprotein: characteristics of N-linked sugar chains of glycoproteins produced in human liver and hepatocellular carcinomas. *Cancer Res* 1993;53:2970-5.

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Anti-GM1 antibodies in polyneuropathies of unknown origin

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Abstract

This study was undertaken to determine whether anti-GM1 titres are raised in polyneuropathies of unknown origin and whether determination of these titres is useful for diagnosing these conditions. The study population comprised 20 controls (aged 36-88 years), 12 patients with polyneuropathies of known origin (aged 31-81 years) and 15 patients with polyneuropathies of unknown origin (aged 40-77 years). Antibody levels were measured using a commercial GM1 enzyme linked immunosorbent assay kit (Buehlmann

Laboratories). Mean anti-GM1 IgG and IgM antibody titres were not raised in patients with polyneuropathies of unknown origin. Anti-GM1 IgG antibody titres were raised in one and GM1 IgM antibody titres in none of the patients with polyneuropathies of unknown origin. In conclusion, GM1 antibody levels are rarely raised in polyneuropathies of unknown origin and probably play a minor role in the pathogenesis of these conditions.

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Keywords: ganglioside antibodies, polyneuropathies, motor neuron disease, neuroimmunology.

Table 1 Clinical, electrophysiological, bioptical, and immunological findings in patients with a polyneuropathy of unknown origin

Patient initials	Sex	Age (years)	Motor impairment	Sensory impairment	Electroneurography	Electromyography	Biopsy	GM1 IgG titre (%)	GM1 IgM titre (%)
BE	F	56	Yes	Yes	1	2	3	2.0	3.0
CH	F	77	Yes	Yes	1	3	2	1.3	9.0
FR	M	46	Yes	Yes	1	3	4	26.0*	15.0
HA	M	52	Yes	No	2	1	1	2.0	6.0
KR	M	53	Yes	Yes	3	3	4	0.6	4.5
KG	M	40	Yes	Yes	1	1	4	2.0	3.0
KE	M	67	Yes	Yes	3	3	4	2.0	3.0
PH	M	47	No	Yes	3	1	2	4.0	6.0
PJ	M	65	Yes	Yes	2	2	4	7.0	4.0
PR	M	52	Yes	No	2	3	2	2.0	10.0
PR	M	60	Yes	No	1	3	1	3.0	18.0
SM	F	65	Yes	Yes	2	1	4	5.2	6.0
SE	M	76	Yes	No	1	2	4	2.0	3.0
SR	M	60	Yes	Yes	1	3	4	4.0	3.0
WM	F	50	No	Yes	1	3	4	0.0	15.0

Electroneurography score: 1 = unclassified; 2 = axonal; 3 = demyelinating. Electromyography score: 1 = neurogenic; 2 = normal; 3 = not applied. Biopsy score: 1 = axonal; 2 = demyelinating; 3 = mixed; 4 = not applied. * Increased antibody titre.

There is increasing evidence that some neuropathies are immune mediated, are often demyelinating in nature, and respond to immune modulating therapy.¹ In these autoimmune neuropathies serum antibodies directed against epitopes of glycoproteins or glycosphingolipids, or both, are frequently detected, but can appear in patients with other neurological diseases and also in healthy subjects.²

Increased anti-GM1 IgM antibody titres are predominantly associated with chronic motor neuropathies, whereas anti-GM1 IgG antibodies are mainly found in acute and chronic axonal motor neuropathies.³ The presence of these antibodies raised the possibility that serum testing could provide diagnostic support and aid patient management.³

The presence and pathogenetic role of anti-GM1 antibodies in polyneuropathies of unknown origin, which represent 10 to 15% of all polyneuropathies, is uncertain. In this prospective open trial, we aimed to determine whether anti-GM1 antibody titres are raised in polyneuropathies of unknown origin and whether determination of these titres is useful for diagnosing these conditions.

Methods

The study population comprised 20 healthy subjects (12 women and eight men), aged 36–88 years, 12 patients (four women and eight men) with a polyneuropathy of known origin (diabetic (n=7), mitochondrial (n=1) and polyradiculitis (n=4)), aged 31–81 years, and 15 patients (four women and 11 men) with a polyneuropathy of unknown origin, aged 40–77 years. Clinical, electroneurographic, electromyographic, and bioptical findings in patients with polyneuropathies of unknown origin are listed in table 1. Informed consent for the procedure was obtained from all subjects.

ANTI-GM1 ANTIBODY TESTING

A 2 ml blood sample was taken from each patient into a standard venepuncture tube without anticoagulant. The blood samples were left standing for two to three hours at room temperature and were then centrifugated at 4°C. Specimens were stored at –30°C pending analysis. Samples were assayed in duplicate

using a commercially available anti-GM1 enzyme linked immunosorbent assay kit (EL-GM1), kindly provided by Buehlmann Laboratories, Allschwil, Switzerland. Serum, pre-diluted 1 in 50 with a buffered saline solution, was incubated in GM1 coated microtube wells. A horseradish peroxidase labelled antibody directed against human IgG or IgM was then added. Absorbance was measured at 492 nm. Results were expressed as the ratio in per cent between the blank corrected average absorbance of the sample and the blank corrected average absorbance of the positive control, as recommended by the manufacturer.⁴ The positive controls comprised a 1300 titre serum for GM1 IgM and a 4000 titre serum for GM1 IgG.

STATISTICS

Differences in the mean age between the groups and in the antibody titres between the sexes were calculated using the Student's *t* test for continuous variables. As the patients' antibody titres were not normally distributed, the non-parametric Mann-Whitney U-test was applied to compare antibody titres between groups; *p* values (two-tailed) less than 0.05 were considered significant.

Results

CONTROLS

Anti-GM1 IgG and anti-GM1 IgM antibody titres did not differ significantly between the sexes (*p*=0.693 and *p*=0.132) and, therefore, results were calculated for the control group as a whole. The mean (2SD) age of the controls was 60.5 (28.9) years. The mean (2SD) anti-GM1 IgG titre was 4.0% (6.9%) (fig 1). The anti-GM1-IgG titre for one subject only was outside the upper reference limit (fig 1). The mean (2SD) anti-GM1 IgM titre was 10.3% (15.3%) (fig 1). One subject (not the one with the increased anti-GM1 IgG titre) had an anti-GM1 IgM titre outside the upper reference limit (fig 1).

PATIENTS WITH POLYNEUROPATHIES OF KNOWN ORIGIN

The mean age of patients with polyneuropathies of known origin was 65.9 (27.4) years,

Figure 1 Anti-GM1 IgG and IgM antibody titres in controls and patients with polyneuropathies of known and unknown origin, showing normal means and the upper reference limits. Two patients with diabetic polyneuropathies had raised anti-GM1 IgM antibody titres (○). These patients did not exhibit the clinical signs of polyradiculitis or multifocal motor neuropathy.

very similar to that of the controls ($p=0.303$). The mean anti-GM1 IgG and anti-GM1 IgM antibody titres were, respectively, 4.8 (8.5%) and 12.7 (40.4%). These values were not significantly different ($p=0.63$ and $p=0.26$) from those found in controls (fig 1). One patient with a diabetic polyneuropathy had an increased anti-GM1 IgG antibody titre and two others with diabetic polyneuropathies had raised anti-GM1 IgM antibody titres (fig 1). None of these patients exhibited the clinical signs of polyradiculitis or multifocal motor neuropathy. Electroneurographic investigations in these patients revealed a mixed type polyneuropathy with no evidence of a conduction block. Mean anti-GM1 IgG and anti-GM1 IgM antibody titres were within the normal range in patients with diabetic polyneuropathy, polyradiculitis and mitochondrial myopathy.

PATIENTS WITH POLYNEUROPATHIES OF UNKNOWN ORIGIN

The mean age of patients with polyneuropathies of unknown origin was 57.7 (21.5) years, which was not significantly different from that of controls ($p=0.538$) or those with polyneuropathies of known origin ($p=0.094$). Mean anti-GM1 IgG and anti-GM1 IgM antibody titres were, respectively, 4.2% (12.6%) and 7.2% (10.2%). These values were not significantly different from those recorded for controls ($p=0.663$, $p=0.246$) and for patients with polyneuropathies of known origin ($p=0.204$, $p=0.636$; fig 1). None of the patients had raised anti-GM1 IgM antibody titres and only one patient had a raised anti-GM1 IgG antibody titre (fig 1). This patient had a mixed type polyneuropathy (table 1).

Discussion

In the present study we found that in patients with polyneuropathies of unknown origin the anti-GM1 IgM antibody titres were within the normal range while the anti-GM1 IgG antibody titre was raised in one patient only. This contrasts with the findings reported by other authors, who observed that anti-GM1 antibody titres were raised to varying degrees in several neuropathies.¹⁴ Low and intermediate anti-GM1 antibody titres are common in healthy subjects and in patients with various motor neuropathies, but lack specificity.^{3,4} High titres have been observed in some patients with multifocal motor neuropathy, Guillain Barré syndrome, gammopathy, and lower motor neuron disease.^{3,4} In polyneuropathies anti-GM1 antibody titres are reported to be normal or slightly increased.³ Unfortunately, variable results and the overlap in data among the disease categories makes interpretation of the findings difficult.¹

At present, it is not clear whether anti-GM1 antibodies are a pathogenetic manifestation of an ongoing autoimmune process in acute and chronic neuropathies or whether they are only an associated epiphenomenon,¹⁴ and therefore whether they can be used as a marker of disease or to predict neuronal damage. The latest findings in demyelinating polyneuropathies suggest that anti-GM1 antibodies are not responsible for the primary pathogenetic process but interfere with remyelination of demyelinated axons, block sodium channels and prevent their reorganisation.⁵

Many arguments have been put forward in favour of a pathogenetic role for anti-GM1 antibodies in immune mediated neuropathies. (1) The clinical response and decrease in anti-GM1 antibody titres during immune modulating treatment.⁶ (2) The induction of myelin splitting, demyelination and conduction block by the injection of human serum containing anti-GM1 antibody into the rat sciatic nerve.⁷ (3) The induction of peripheral neuropathies with conduction block by chronic injection of GalNAc epitopes and anti-GM1 antibodies into rabbits. (4) The presence of para and internodal anti-GM1 antibody deposits in some neuropathies.⁶ (5) Localisation of the cholera toxin, which binds specifically to GM1 sites, at the paranodal sheath and of the peanut agglutinin, which recognises glycoproteins that bear Gal(β1-3)GalNAc epitopes, at the nodal gap extending to the surface of adjacent Schwann cells.⁸ (6) Anti-GM1 antibodies disrupt myelination in the neonatal rat spinal cord.⁶ (7) Anti-GM1 antibodies can kill spinal neurons in vitro.

Conversely, there are also many arguments against a pathogenetic role for anti-GM1 antibodies in autoimmune neuropathies. (1) These antibodies are present in a wide variety of other diseases and also in healthy subjects.⁹ (2) There is no correlation between the antibody titres and clinical recovery following immune modulating therapy.⁵ (3) Injections of patient serum with high anti-GM1 antibody titres do not necessarily induce demyelination in rats.¹⁰ (4)

Patients with normal anti-GM1 antibody titres also respond to immune modulating therapy.

There are three possible reasons for the normal anti-GM1 antibody titres observed in our patients: firstly, the polyneuropathies of unknown origin investigated might not be immune mediated¹¹; secondly, these polyneuropathies might be immune mediated but induced by antibodies directed against epitopes other than those of gangliosides; and thirdly, the assay applied was of low sensitivity and specificity and may not have recognised disease specific anti-GM1 antibodies. Other authors, however, have detected raised anti-GM1 antibody titres using the EL-GM1 assay and, hopefully, the specificity of anti-GM1 antibody testing will be increased by analysing GM1 target epitopes other than Gal(β1-3)GalNAc.⁹

In conclusion, our results suggest that quantification of anti-GM1 antibody titres is of little help in the diagnosis of polyneuropathies of unknown origin.

1 Kornberg AJ, Pestronk A. The clinical and diagnostic role of anti-GM1 antibody testing. *Muscle Nerve* 1994;17:100-4.

- 2 Bansal AS, Abdul-Karim B, Malik RA, Goulding P, Pumphrey RS, Boulton AJ, *et al.* IgM ganglioside GM1 antibodies in patients with autoimmune disease or neuropathy, and controls. *J Clin Pathol* 1994;47:300-2.
- 3 Kornberg AJ, Pestronk A. The clinical correlates of high-titer anti-GM1 antibodies. *Ann Neurol* 1994;34:234-7.
- 4 Pestronk A. Invited review: motor neuropathies, motor neuron disorders, and antiglycolipid antibodies. *Muscle Nerve* 1991;14:927-36.
- 5 Kaji R, Hirota N, Oka N, Kohara N, Watanabe T, Nishio T, *et al.* Anti-GM1 antibodies and impaired blood-nerve barrier may interfere with remyelination in multifocal motor neuropathy. *Muscle Nerve* 1994;17:108-10.
- 6 Thomas FP, Trojaborg W, Nagy C. Experimental autoimmune neuropathy with anti-GM1 antibodies and immunoglobulin deposits at the nodes of Ranvier. *Ann Neurol* 1991;82:378-83.
- 7 Santoro M, Uncini A, Corbo M, Staugaitis SM, Thomas FP, Hays AP, *et al.* Experimental conduction block induced by serum from a patient with anti-GM1 antibodies. *Ann Neurol* 1992;31:385-90.
- 8 Corbo M, Quattrini A, Latov N, Hays AP. Localisation of GM1 and Gal(β1-3)GalNAc antigenic determinants in peripheral nerve. *Neurology* 1993;43:809-14.
- 9 Willison HJ, Paterson G, Kennedy PG, Veitch J. Cloning of human GM1 antibodies from motor neuropathy patients. *Ann Neurol* 1994;35:471-8.
- 10 Harvey GK, Toyka KV, Zielasek J, Kiefer R, Simons C, Hartung HP. Failure of anti-GM1, IgG or IgM to induce conduction block following intraneural transfer. *Muscle Nerve* 1995;18:388-94.
- 11 Willison HJ, Chancellor AM, Paterson G, Veitch J, Singh S, Whitelaw J, *et al.* Antiglycolipid antibodies, immunoglobulins and paraproteins in motor neuron disease: a population based case-control study. *J Neurol Sci* 1993;114:209-15.

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Quantification of HIV DNA in the brain by PCR: differences between fresh frozen and formalin fixed tissue

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Abstract

HIV-1 DNA extracted from frozen and formalin fixed brain tissue can be detected using PCR. This work has been extended by amplifying, using semiquantitative PCR, HIV DNA extracted from frontal lobe tissue of 16 patients with AIDS (eight positive and eight negative for p24 antigen). DNA was amplified using HIV-1 *pol* gene digoxigenin labelled primers and detected by chemiluminescence and densitometry. Cloned standards were amplified in parallel for quantification. HIV DNA levels detected in frozen tissue showed a correlation with p24 positivity and the severity of the histological diagnosis. This correlation was less clear in the formalin fixed material.

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Keywords: HIV, PCR, frozen tissue, formalin fixed tissue.

About 30% of adult patients and 50% of paediatric patients with AIDS are likely to develop symptoms encompassed by the term HIV as-

sociated dementia complex (HIV-ADC) and it is probable that these percentages would be greater if patients did not succumb to earlier complications. The HIV-ADC includes a wide range of neurological problems in which the pathogenetic processes involved are not fully understood. There are two likely pathogenetic mechanisms, both of which may be contributing to disease. Firstly, the direct cytopathic effects of viral infection may be responsible for the cell destruction and loss of neurons which are normally observed in HIV-ADC. This type of pathogenesis may be important during the terminal stages of AIDS.¹ However, neurons *in vivo* show no evidence of HIV infection while microglia and macrophages are typically infected and the formation of multinucleated giant cells (MGC) is a diagnostic feature of HIV encephalitis (HIVE) and HIV leucoencephalopathy (HIV lep).¹ Secondly, damage is the indirect consequence of HIV infection and is probably macrophage mediated. Neuropathological changes are the result of secretion of neurotoxic factors including arachidonic acid, cytokines and toxic oxygen metabolites.² Several reports have im-

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