

## SHORT REPORT

## Antiganglioside antibodies in paraneoplastic peripheral neuropathies

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A total of 29 patients with cancer and neuropathies of unknown origin that were possibly paraneoplastic were tested for antiganglioside antibodies by immunodot blot and ELISA. None of the patients had onconeural antibodies. They were compared with 41 normal subjects and 187 patients with metabolic or idiopathic neuropathies. Antiganglioside antibodies, mainly IgM anti-GM1, were more frequently found in the patients with cancer than in the control groups. However, the levels of antibodies were not different from those of the controls. There was no correlation with the pattern of the neuropathy. These results do not support the hypothesis that antiganglioside antibodies are frequent and major immunological targets in paraneoplastic neuropathies.

Gangliosides, a complex family of sialylated glycosphingolipids, are components of the cell membrane that are particularly concentrated in the peripheral nervous system where several of them may be target antigens in immune mediated neuropathies.<sup>1</sup> Tumour cells over-express a great variety of gangliosides, and aberrant glycosylation is a frequent mechanism in cancerogenesis leading to the formation of gangliosides that are not expressed by the mature normal cells from which the tumour develops.<sup>2–9</sup> Several of these gangliosides are shared with the nervous system such as GM1, GM2, GM3, GD2, and GD3. Abnormal production of gangliosides occurs in a wide range of cancers such as melanoma, small cell lung cancer (SCLC), neuroblastoma, hepatoma, sarcoma, and kidney and colon carcinoma. In addition, several of these tumours are known to deliver gangliosides to the blood circulation.<sup>6–8</sup> Both in the blood and at the cell surface, gangliosides can be recognised by the immune system<sup>10</sup> so that they are potential targets for anticancer immunisation.<sup>11</sup> Alternatively, natural immunisation against cancer gangliosides may be at the origin of immune mediated peripheral neuropathies when the epitope is shared by the tumour and the peripheral nervous system. This has recently been reported in at least two patients with melanoma.<sup>12–13</sup> Here we report on a study of antiganglioside antibodies in a series of patients who developed neuropathy in the setting of cancer.

## PATIENTS AND METHODS

## Patients

We studied 29 patients (26 men, 3 women; mean age 67.1 (SD 10.5) years, range 39–85) who developed peripheral neuropathy in the setting of cancer that could not be explained by metastasis, cachexia, treatment toxicity, or cancer related metabolic perturbations (table 1). None of the patients had onconeural anti-Hu, anti-CV2, anti-Yo, anti-Ri, or anti-amphiphysin antibodies. The delay between the onset of the neuropathy and the diagnosis of cancer was below 36 months (median 4 months, mean 9.8 (SD 11.3)). In four

patients, the neuropathy followed the surgical removal of the cancer by a few days to four weeks and in one patient the neuropathy appeared 22 months after the onset of an unremitting malignant lymphoma. The neuropathy was acute in six patients, subacute in 11 and chronic in 12, according to previously published definitions.<sup>14</sup> In four patients the neuropathy was a subacute sensory neuropathy. Eleven patients had a predominantly distal axonal sensory or sensorimotor neuropathy (DASMN). Four had an asymmetrical sensorimotor axonal neuropathy with perivascular inflammatory infiltrates on nerve biopsy in three. One had an acute painful brachial plexopathy. Two patients had demyelinating Guillain-Barré syndrome. In both the tumour was diagnosed during the acute phase of the neuropathy. Five patients had a demyelinating neuropathy consistent with chronic inflammatory demyelinating neuropathy (CIDP) in four. In the last two patients, the neuropathy was axonal and demyelinating. The characteristics of the neuropathy suggested a dysimmune mechanism in at least 14 patients. The tumours present in the patients are listed in table 1. In 28 patients, the cancer was characterised either by computed tomography (CT) scan or by pathological study. One patient had a positive mediastinal positron emission tomography (PET) scan and high blood level of chromogranin suggesting a neuroendocrine cancer, but repeated CT scan was negative.

The sera of 41 normal healthy volunteers and 187 patients with chronic polyneuropathies not associated with cancer were tested by the same methods and these served as controls. These neuropathies were mostly associated with metabolic disorders or were chronic distal axonal neuropathies of unknown aetiology. Patients with monoclonal gammopathies were excluded.

## Methods

Antiganglioside antibodies were detected and characterised by immunodot blot and quantified by enzyme linked immunosorbent assay (ELISA) as previously published.<sup>15</sup> For immunodot blot, purified commercial GM3, GM2, GD3, GM1, GD1a, GD1b, GT1b, and GQ1b gangliosides (Sigma, Saint-Quentin Fallavier, France) were fixed on strips of PVDF-P membranes (Millipore, Saint-Quentin Ivelines, France) and incubated with 1/100, 1/200, and 1/500 dilutions of the patients' sera. Bound antibodies were revealed with alkaline phosphatase conjugated antibodies to human IgG and IgM (Jackson Interchim, Montluçon, France) and NBT/BCIP (Sigma). A clear staining of one or more ganglioside dots at 1/100 or greater dilution was considered as positive.

**Abbreviations:** CIDP, chronic inflammatory demyelinating polyneuropathy; DASMN, distal axonal sensory or sensorimotor neuropathy; ELISA, enzyme linked immunosorbent assay; GBS, Guillain-Barré syndrome; SCLC, small cell lung cancer; SSN, subacute sensory neuropathy

**Table 1** Clinical data and antiganglioside antibody detection in the 29 patients with peripheral neuropathy and cancer

Patient	Sex	Age (years)	Delay (months)*	Evolution	Type	Tumour	Anti-GM1 reactivity	Immunodot-blot titre	ELISA titre	Other reactivity (immunodot-blot)
1	M	72	-36	C	DASMN	Urinary	0			
2	M	85	-9	C	DASMN	Lung (non-SCLC)	0			
3	M	63	-24	C	DASMN	Lung (SCLC)	0			
4	M	39	+0.5	A	DASMN	Thyroid	0			
5	M	77	-1	A	Plexopathy	Thymus	0			
6	M	73	-26	C	SSN	Lung (SCLC)	0			
7	M	78	-2	SA	Axonal and demyelinating	Prostate	0			
8	M	65	+0.5	SA	Asymmetrical axonal neuropathy†	Tongue	+	1/100	2	
9	M	51	-2	SA	DASMN	Metastatic adenocarcinoma	+	1/100	2	
10	F	59	-7	A	SSN	PET mediastinal fixation	0			
11	M	61	-6	C	CIDP	Liver	+	1/500	7.1	GM2 (1/100)
12	M	84	-7	SA	Asymmetrical axonal neuropathy	Lung	+	1/200	3.9	
13	M	74	-4	C	Asymmetrical axonal neuropathy†	Urinary	+	1/200	3.3	GM3 (1/200)
14	M	61	-1	A	DASMN	Stomach	0			
15	F	66	-36	SA	CIDP	Malignant lymphoma	0			
16	F	73	-26	C	DASMN	Pancreas	+	1/200	2.2	
17	M	75	-24	C	DASMN	Kidney	+	1/500	10	GD1b (1/100)
18	M	73	-3	SA	CIDP	Pancreas	0			
19	M	75	+2	SA	DASMN	Tonsil	0			
20	M	57	-12	SA	SSN	Lung	0			
21	M	48	-3	SA	Demyelinating neuropathy	Melanoma	0			
22	M	73	-4	C	CIDP	Colon	+	1/200	6	
23	M	65	-3	SA	SSN	Lung	0			
24	M	70	+3	C	DASMN	Malignant lymphoma	0			
25	M	72	+22	C	DASMN	Malignant lymphoma	+	1/500	5.9	
26	M	73	+0.5	SA	Axonal and demyelinating	Colon	0			
27	M	66	-1	A	GBS	Colon	0			
28	M	65	-18	C	Asymmetrical neuropathy†	Malignant lymphoma	0			
29	M	54	-0.5	A	GBS	Tongue	0			

\*Positive number indicates that the neuropathy preceded the cancer diagnosis and negative number that the neuropathy followed the diagnosis of cancer. In all the cases the neuropathy and the cancer were simultaneously present at least at one time.

†Perivascular inflammatory infiltrates on nerve biopsy.

A, acute; C, chronic; CIDP, chronic inflammatory demyelinating polyneuropathy; DASMN, distal axonal sensory/sensorimotor neuropathy; F, female; GBS, Guillain-Barré syndrome; M, male; SA, subacute; SSN, subacute sensory neuropathy; SCLC, small cell lung cancer.

Antiganglioside GM1 antibodies were quantified by ELISA using Costar ELISA plates (Biomedical Diagnostic, Marnes la Vallée, France) coated with GM1. The patients' sera were tested at 1/20 dilution. Antibody binding was detected with peroxidase conjugated goat antihuman IgG and IgM (IGN Biomedical). As control, two positive and eight negative sera were tested in duplicate in the same plate. For all sera, serum blanks were tested for subtraction of non-specific signals to eliminate false positive results. Titres of two or more were considered significantly outside the normal range (0–2).

## RESULTS

Among the 29 patients with neuropathy and cancer, nine (31%) had polyclonal IgM serum antibodies that reacted with GM1 ganglioside of which three also reacted with GM2, GM3, or GD1b (table 1). On immunodot blot, immunoreactivity was observed at 1/100 dilution in two patients, at 1/200 dilution in four, and at 1/500 or more in three. On ELISA, the same nine sera only were positive for IgM anti-GM1 antibodies. There was a good correlation between the two methods, the highest titre on ELISA (5.9–10) being observed with the sera positive at >1/500 dilution on immunodot blot and the lowest (1.9–2.2) with those positive at 1/100. Antiganglioside antibodies were not associated with any particular type of tumour or neuropathy. The three patients with the highest anti-GM1 antibody titre had CIDP and liver carcinoma, DASMN and lymphoma, and DASMN and kidney adenocarcinoma, respectively. Among the 187 patients with axonal neuropathy of unknown aetiology, 27 (14.4%) had

antiganglioside antibodies and there was five positive sera among the 41 normal subjects (12.2%). In the two control groups, the antibodies were of IgM isotype and reacted with GM1 ganglioside. Some sera also reacted with GD1b or GM2. On immunodot blot the titres were between 1/100 and 1/200 and on ELISA between 2 and 4. The proportion of patients with antiganglioside antibodies was significantly higher among the patients with neuropathy and cancer than in the two control groups ( $\chi^2$  test;  $p = 0.026$  and  $p = 0.05$ ) while it was not significantly different between the two control groups.

## DISCUSSION

Peripheral neuropathies occurring in the setting of cancer, whose presence can not be explained by the usual causes of cancer associated neuropathies give rise to the question of whether they are paraneoplastic. Onconeural antibodies are detected in a minority of these neuropathies,<sup>14</sup> but for the rest there are no known markers at present. Whether these neuropathies result from chance association or depend on immunological mechanisms induced by the tumour is still unknown. In the present study, the patients who developed peripheral neuropathy in the setting of cancer were more frequently found to have antiganglioside antibodies than patients with a neuropathy of unknown origin or the normal subjects. However, the pattern of the antibodies was the same in the three groups, consisting mainly of IgM anti-GM1 and occasionally anti-GM2, GM3, or GD1b antibodies. Patients with cancer have been demonstrated to harbour IgM

antiganglioside antibodies more frequently and with higher levels than normal subjects probably as a result of an antitumour immune reaction.<sup>10</sup> The increased frequency of antiganglioside antibodies in patients with cancer and neuropathy may result from the same mechanism.

Antibodies to a wide range of gangliosides have been associated with different forms of acute or chronic peripheral neuropathies. Although it is still difficult to correlate an antiganglioside antibody pattern with a clinically and electrophysiologically characterised neuropathy, several syndromic associations have been identified.<sup>1</sup> Anti-GM1 and other antibodies to mono or disialylated gangliosides occur in patients with motor neuropathies while antibodies to disialylated gangliosides including GD1b, GD3, and GQ1b are associated with ataxic neuropathies and cranial nerve involvement. In our series of patients with cancer, IgM antiganglioside antibodies were not associated with a particular form of neuropathy even in those with the highest titres.

Our results do not support the hypothesis that the antiganglioside antibodies detected in our patients were responsible for their neuropathies. Gangliosides are probably not a frequent and major immunological target in patients with cancer associated neuropathies in whom no anti-onconeural antibodies are detected. Cancer associated neuropathies for which antibodies to gangliosides occur with a relatively good correlation between the pattern of the neuropathy and the antiganglioside antibody profile<sup>11, 12</sup> probably represent a minority of cases and need further investigation. In patients with cancer, antiganglioside antibodies reflect an antitumoural immune reaction. Although they were probably not responsible for the neuropathies in our patients, their presence may provide indirect support for an argument in favour of an autoimmune mechanism directed towards as yet unknown onconeural antigens in patients with cancer and neuropathies of unknown origin.

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