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ANTIGANGLIOSIDE ANTIBODIES IN SERUM OF PATIENTS WITH CHRONIC SENSORY ATAXIC NEUROPATHY

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Abstract Recent studies have shown that antiganglioside antibodies, particularly those associated with the disialosyl group, may be involved in immune-mediated sensory peripheral neuropathies. We report the results of plasma screening for antiganglioside antibodies in two patients with chronic ataxic neuropathy. We found reactivity against gangliosides GD3, GD1b, and GT1b in one of them and against GD1a in the other, even though both had nearly identical clinical pictures. Results suggest that anti-GD1a antibodies, which are usually associated with motor polyneuropathy, may also be involved in the pathogenesis of clinically pure sensory polyneuropathy.

Key words: chronic sensory ataxic neuropathy, anti-ganglioside antibody, b-series ganglioside, anti-GD1a antibody

Resumen ***Anticuerpos anti-gangliósidos en pacientes con neuropatía sensitiva atáxica crónica.*** Estudios recientes han demostrado que los anticuerpos anti-gangliósidos que contienen un grupo disiálico en su molécula están relacionados con la patogénesis de las neuropatías periféricas sensitivas. Comunicamos los resultados obtenidos en la búsqueda en plasma de anticuerpos anti-gangliósidos en dos pacientes con neuropatía atáxica crónica. En uno de ellos encontramos reactividad contra los gangliósidos GD3, GD1b y GT1b y en el otro anticuerpos anti-GD1a. Ambos presentaban el mismo cuadro clínico. Estos resultados sugieren que los anticuerpos anti-GD1a, que habitualmente están asociados a neuropatías motoras, también podrían participar en la patogenia de las neuropatías clínicamente sensitivas.

Palabras Clave: neuropatía atáxica sensitiva crónica, anticuerpos anti-gangliósidos, gangliósidos serie b, anticuerpos anti-GD1a

Monoclonal IgM antibodies that recognize gangliosides containing a disialosyl group were first described by Ilyas et al.[1] in a patient with a predominantly sensory demyelinating neuropathy. The monoclonal IgM reacted primarily with GD3 and GT1b gangliosides, but also showed strong cross-reactivity with GD1b and GD2. Other patients with predominantly sensory neuropathy and monoclonal IgM antibodies to one or more gangliosides of the same series have been reported since then[2], indicating that serum antiganglioside antibodies, particularly those associated with the disialosyl group and reacting with GD3, GD1b or GT1b gangliosides, may be involved in immune-mediated sensory neuropathies. We now report findings on antiganglioside antibodies in the plasma of two patients with chronic sensory ataxic neuropathy. A preliminary communication has been presented elsewhere[3].

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Material and Methods

Patients

Case 1. A 37-year old man was evaluated because of a 3-year history of imbalance and numbness in all limbs. Numbness and burning pain had begun in his left foot and spread insidiously to the right foot and upper limbs during the 6 months prior to consultation. He had a severe gait impairment, adopting a wide base and taking slow steps; the feet were thrust out with each step and the sole of the foot struck the floor forcibly. He denied weakness, and had no family history of neuropathy. Physical examination revealed symmetrical sensory loss for all modalities (mainly vibration and propioception) in the distal segments of the four limbs. Romberg's sign was positive, and tendon reflexes were absent. Muscle strength and bulk were normal. Cranial nerve functions were spared. The spinal fluid had a protein concentration of 0.68 g/L (normal: < 0.40 g/L) and was acellular. Protein monoclonal bands were not found by immunoelectrophoresis.

The patient's plasma was tested for antiganglioside antibodies employing high performance thin-layer chromatography (HPTLC)-immunostaining (see below). Concentric needle electromyography (EMG) was performed in proximal and distal muscles of the four limbs and in both masseters and orbicularis oculi muscles. No spontaneous activity was seen at rest and a full interference pattern was obtained in each

TABLE 1.– *Nerve conduction studies*

Nerve	Patient 1		Patient 2		Control Ranges	
	R	L	R	L		n
Motor nerve conduction velocity (m/s)						
Median	57	52	50	70	50 - 67	45
Ulnar	52	66	59	50	51 - 72	51
Peroneal	40	42	39	35	42 - 62	45
Distal Latency (ms)						
Median	3.5	3.0	4.9	5.6	2 - 4	53
Ulnar	3.1	3.1	5.0	4.8	1.5 - 3.5	48
Peroneal	5.8	6.4	6.7	8.4	3 - 5	30
Amplitude (mV)						
Median	10	12	10	12	5 - 15	56
Ulnar	8	9	5.0	4.8	5.2 - 31	80
Peroneal	4	3	6	6	2.2 - 14	42
F wave latency (ms)						
Median	45	30	66	69	26 - 32.6	33
Ulnar	37	32	67	80	25 - 33	33
Peroneal	60	71	61	65	34 - 51	40
Sensory nerve conduction velocity (m/s)						
Median	AB	58	AB	AB	44 - 64	40
Sural	35	AB	AB	AB	42 - 52	40
Amplitude sensory potential (uV)						
Median	AB	10	AB	AB	5 - 65	40
Sural	4	AB	AB	AB	9 - 26	40

mV: milivolts; ms: miliseconds; μV: microvolts. AB: absent. R: right; L: left; n: number of subjects; m/s: meters per seconds. F wave was obtained by stimulating distally the corresponding motor fibers at the median, ulnar and deep peroneal nerves.

muscle during sustained voluntary contraction; most of the single motor unit potentials (MUAP) were bi- or triphasic and had normal duration and amplitude. Nerve conduction studies demonstrated slightly reduced motor conduction velocity of the right peroneal nerve, absence of sensory potentials in right median and left sural nerves, prolonged distal motor latencies in both peroneal nerves, and delayed F waves in muscles of the upper and lower limbs (Table 1). A sural nerve biopsy was obtained. A fragment of the nerve was formalin fixed and paraffin embedded for routine examination. Hematoxylin-eosin, PAS, and Congo Red techniques were performed in the specimen. A portion of biopsied sural nerve specimen was fixed overnight in 3% gluta-raldehyde in 0.125 M cacodylate buffer (pH 7.4), washed, osmicated, and plastic embedded. The other portion of the specimen was fixed in the same glutaraldehyde solution, washed, and osmicated for teased-fiber analysis. Semithin sections were made for light microscopy and ultrathin sections were made for electron microscopy. Density and fiber size distribution of myelinated and unmyelinated fibers were determined. Sural nerve biopsy revealed severe demyelination and 90% reduction of large-diameter fibers, with absence of inflammatory cells and presence of axonal degeneration.

Case 2. A 39-year old man presented with a 9-year history of imbalance and numbness, pain, and paresthesias in both feet and hands. He denied weakness, and had no family history of neuropathy. Neurological examination showed normal strength and muscle bulk, tendon reflexes were absent, severe impaired vibration sense was detected at ankles and toes, touch and pain sensations were also mildly impaired in the distal segments of the four limbs. When walking, he adopted a wide base, took slow steps, and needed to watch his steps on the floor. Romberg's sign was positive. Cranial nerves were normal. The spinal fluid had a protein concentration of 1.10 g/L and was acellular. Protein monoclonal bands were not found. Plasma was tested for antiganglioside antibodies employing HPTLC-immunostaining (see below). EMG with coaxial needle electrodes was carried out in proximal and distal muscles of the four limbs and in both masseters and orbiculari oculi muscles; no spontaneous activity was detected at rest. On voluntary contraction a full interference pattern was observed in all muscles tested, except in the left tibialis anterior where a slightly reduced interference pattern was recorded. MUAPs were bi- or triphasic and had normal duration and amplitude; increased numbers of fragmented and polyphasic MUAPs were seen only in the left tibialis anterior muscle. Nerve conduction studies showed absence of sensory potential in both median and both sural nerves, slight reduction of motor fiber conduction velocity in both peroneal nerves, and prolonged F waves in muscles of the upper and lower limbs (Table 1). A sural nerve biopsy was performed and treated as in patient 1. The specimen revealed severe demyelination and 35% reduction of large diameter fibers. No inflammatory cells and no signs of axonal degeneration were seen.

Inmunological studies

The glycolipids used in this study were purified by DEAE-Sephadex chromatography and HPLC on latrobeads columns. They were isolated from the following sources: GM1, GD1a, GD1b, GT1b, GQ1b, sulfatide from human brain, GM2 from Tay-Sachs brain, GM3 from dog erythrocytes, GD3 from chicken brain, and LM1 from human erythrocytes. Asialo-GM1 (GA1) was prepared by acid hydrolysis of cow brain gangliosides. The glycolipid mixture was separated on HPTLC plates in running solvent chloroform-methanol-aqueous 0.2% $CaCl_2$ (45:45:10) using a tank designed to obtain highly reproducible chromatograms[4]. The plates were air dried, coated by dipping for 1 min in a 0.5% solution of polyisobutylmethacrylate (Plexigum P 28, Röhm and Haas, Darmstadt, Germany) in n-hexane-chloroform (9:1), air dried again, blocked with BSA-PBSt (1% BSA in PBS containing 0.05% Tween 20) for 1 hr, incubated overnight with BSA-PBSt diluted plasma (1/20), and washed thoroughly with PBSt. Binding was detected following a 2 hr incubation period with BSA-PBSt diluted (1/1000) peroxidase-conjugated rabbit anti-human IgM and IgG antibodies (Dakopatts, Hamburg, Germany). All the incubation steps were performed at 4°C. After washing, color development was achieved in a substrate solution containing 2.8 mM 4-chloro-1-naphthol and 0.01% H_2O_2 in methanol-20 mM Tris-HCl buffer, pH 7.4 (1:29). The reaction was stopped after 30 min by washing the plates with PBSt. Binding inhibition by soluble antigen was accomplished by incubating the plasma with a 0.1 mM solution of glycolipid for 1 hr before addition to the plates.

The plasma of patient 1 showed IgM-reactivity against GD3, GD1b, and GT1b (Fig. 1B). The antibody binding was abolished completely by preincubation of plasma with soluble GD3, GD1b, or GT1b (data not shown), indicating a homogeneous and cross-reacting population of antibodies. In contrast, the plasma of patient 2 contained antibodies of IgG isotype reacting with GD1a ganglioside (Fig. 1C). To confirm the specificity of these antibodies, pure GD1a was chromatographed in two additional solvent systems and immunostained as described above. In both cases a perfect fit between orcinol staining and immunospot was found (data not shown), confirming that GD1a and not a contaminant was the antigen. Antibodies with the specificity found in these two patients were not detected in normal human plasma (data not shown)[4].

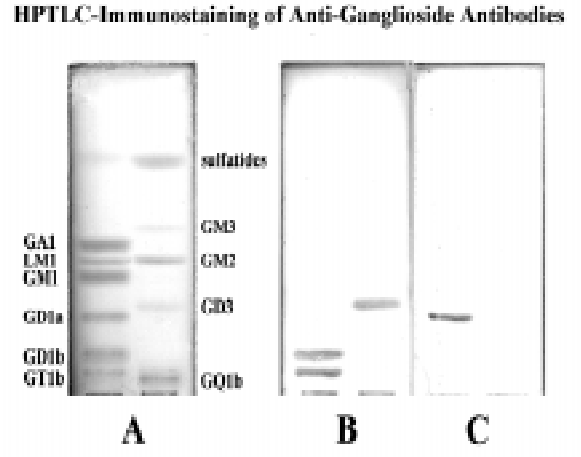

Fig. 1.– Anti-ganglioside antibodies in patients' plasma. A glycolipid library con-taining GM1, GM2, GM3, GD1a, GD1b, GD3, GT1b, GQ1b, GA1, LM1, and sulfatides was separated on HPTLC plates and incubated with 1:20 dilution of patient 1 (B) and patient 2 (C) plasma as described in the text. Binding of IgM (B) and IgG (C) was detected using specific peroxidase-labeled secondary antibodies. One plate was stained with orcinol reagent for chemical detection of gangliosides (A).

Discussion

There were striking clinical, pathological, and electrophysiological similarities between our two patients. Their main complaint was sensory impairment, which was greater in the first patient than the second. Gait disturbance and lack of tendon reflex were found in both. Both had normal muscle strength and bulk, and increased concentration of spinal fluid proteins.

Nerve biopsies revealed loss of fibers and demyelination in both samples, but axonal degeneration was seen only in patient 1. Neither onion bulbs nor inflammatory cells were found in either patient.

Normal EMG patterns were observed for both patient 1 and patient 2 in all the muscles tested, with the exception of the left tibialis anterior of patient 2 where a slightly diminished interference pattern was obtained. However, mild impairment of motor nerve conduction parameters was observed on electrophysiological examination of their mixed nerves, indicating that slight demyelination of the motor fibers was also part of the pathological condition. This interesting observation raises a question regarding classification of our patients' conditions, is it possible to consider from the clinical viewpoint as a "pure" sensory polyneuropathy disease which also shows impairment of motor nerve fiber conduction on laboratory testing? Furthermore, is this entity part of a wide spectrum of immunomediated neuropathies where both sensory and motor fibers are involved with varying intensities? The predominant or unique clinical manifestations may be motor signs in some cases and sensory features in others (such as these two patients). Our current knowledge of such diseases does not allow a definitive answer. On clinical grounds, our patients might be considered to have a "pure" sensory neuropathy.

Patient 1 did not show presence of IgM paraprotein, an observation at variance with most reports on sensory ataxic neuropathy[2]. This suggests that these antibodies are polyclonal, as is the case with some patients with multifocal motor neuropathy, having anti-GM1 antibodies[5]. However, the possibility cannot be excluded that a monoclonal band may have been present at a concentration below the detection threshold of the technique employed.

The antiganglioside antibodies in the plasma of our two subjects were studied in relation to their pathological conditions, with the following results: The IgM-antibody binding to GD1b, GT1b, and GD3 seen in patient 1 was

inhibited by preincubation of serum with a 0.1 mM solution of any of these three gangliosides (data not shown). The results suggest that a similar epitope shared by the gangliosides was involved in binding of the antibodies. The common structural feature of the gangliosides recognized by the three antibodies was a 2-8-linked disialosyl group on the proximal galactose residue of the oligosaccharide core ("b-series gangliosides"). Antibodies reacting with b-series gangliosides bound to nerve fibers in sensory neuropathies have been reported by various authors, and the disialosyl group has been proposed as the target antigen for these neuropathies[1, 2].

Patient 2 did not show antibody activity against the b-series gangliosides (GD1b, GD3, or GT1b), but did show abnormal binding of plasma IgG antibodies to GD1a ganglioside. GD1a has structural similarities to GT1b ganglioside, so we look for reactivity of the second patient´s plasma with this ganglioside. We did not find that reactivity, this observation suggested that the second sialosyl group wich is shared by GD1a and GT1b is not the target for this antibody.

High titers of serum antibodies to GD1a were demonstrated in some patients with Guillain-Barre syndrome[6] and in some with prominent motor impairment, such as motor neuron disease and motor neuropathies[7, 8, 9]. Carpo et al.[10] reported a study of 2 patients with pure motor demyelinating neuropathy and high titers of anti-GD1a; interestingly, one of these patients showed lack of sensory action potentials in all the sensory nerves tested, in addition to the expected abnormality of motor conduction velocity.

In conclusion, it appears that different combinations of anti-ganglioside antibodies may underlie the appearance of pure clinical sensory polyneuropathies. Antibodies against GD1b, GD3, and GT1b gangliosides were detected in patient 1, whereas anti-GD1a antibodies were seen in patient 2, despite the fact that these patients had nearly identical clinical pictures.

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It is a capital mistake to theorize before one has data.

Teorizar antes de tener datos es un error garrafal.

Sir Arthur Conan Doyle (1859-1920)

The adventures of Sherlock Holmes, 1891