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LOWER MOTOR NEURON SYNDROME ASSOCIATED WITH ANTI-GM1 ANTIBODIES

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Summary It has been recently recognized that increased titers of serum anti-GM1 antibodies may be associated with motoneurone diseases or with multiple motor neuropathy with or without conduction block and also with chronic sensorimotor neuropathy and Guillain-Barré syndrome. Santoro et al. were the first to note that anti-GM1 antibodies were able to bind to the nodes of Ranvier of the sural nerve of a patient with clinical signs and symptoms mostly resembling amyotrophic lateral sclerosis who also showed, in nerve conduction studies, multifocal motor nerve fibers conduction block and serum IGM anti-GM1 antibodies. The two patients presented in this report had asymetrical motor neurone disease with signs and symptoms of lower motoneurone involvement, and other signs, in the first patient, which suggested the existence of upper motoneurone damage. Besides, the second patient also had clinical sensory impairment in the lower limbs. Electrophysiologically, none of them had nerve conduction block but both showed inexcitable median and sural nerve sensory fibers. Both had high titers of anti-GM1. A sural biopsy of both patients showed immunoglobulins into the sensory fibers. However, we do not know whether the anti-GM1 antibodies bind to a cross-reactive glycolipid other than the GM1 itself. In any case, it seems that the presence of anti-GM1 antibodies might be a marker signalling a potentially treatable immune disorder which may have signs of lower and upper motor neurone disease and, also, clinical and electrophysiological evidences of peripheral sensory involvement.

Key words: motoneurone syndrome, anti-GM1 antibodies

It has been recently recognized that increased titers of anti-GM1 antibodies may be associated with motoneurone disease or with multiple motor neuropathy with or without conduction block and also with chronic sensorimotor neuropathy and Guillain-Barré syndrome[1, 3]. The antibodies react with the GM1 carbohydrate epitopes, which are present in the peripheral and central nervous systems.

Santoro et al.[4] were the first to note that anti-GM1 antibodies were able to bind to the nodes of Ranvier of the sural nerve of a patient with clinical signs and symptoms mostly resembling amyotrophic lateral sclerosis who also showed, in nerve conduction studies, multifocal motor nerve fibers conduction block and serum IgM anti-GM1 antibodies.

Other authors[5, 6] confirmed and extended these observations, showing, experimentally, that IgM anti-GM1 antibodies may bind to motoneurone somae, to the paranodal myelin and to the myelin gap in the node of Ranvier in peripheral nerves. Despite that still is not clear which are the molecular targets of these antibodies, sites of binding were recognized by employing cholera toxin and lecitin peanut agglutinin PNA[6].

In this study we describe two patients with signs and symptoms of lower motoneurones involvement plus features of upper motoneurone

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damage in one of them, and with high titers of sera anti-GM1 antibodies.

Case Reports

Patient 1

A 62 year old man (J.E.), who complained of progressive weakness of his lower limbs, was admitted in the ward for an in-patient searching.

The patient had been feeling pain in his left calf since June, 1991. Later, he began to develop paresthesias and cramps in the left foot and weakness of the whole left lower limb. After 18 months he noted the appearance of carmps in the right lower limb, which became painful and weak. His sphincters and sexual functions were spared.

There was no family history of this or any other neurological disease.

In the clinical examination he was a kind and clever man. His strength was diminished in the left and right wrist extensors (3/5 MRC scale), both psoas were also weak (3/5) as well as the right (4/5) and the left (3/5) quadriceps, both thigh adductors (4/5), both tibialis anterior and peroneal muscles (0/5) which yielded bilateral foot-drop, and both soleus and gastrocnemius muscles (1/5). Mild wasting was observed in the intrinsic hands muscles and severe wasting was found in the peroneal group of both legs. Deep tendon reflexes were brisk, except for the Achilles tendon jerks which were absent. Plantar responses were absent, while hand clonus and Hoffman reflex were present bilaterally. Fasciculations were noted in the tongue, right deltoid and both quadriceps. All modalities of sensation sere spared and the remainder of the neurological and general clinical examinations was normal.

Laboratory testing revealed high titers of antibodies anti-GM1 (patient: 1/6400; controls: < 1/800), (ELISA) without a detectable paraprotein by serum protein electrophoresis. Quantitative IgM, IgG and IgA immunoglobulins were within the normal range as well as glicemia, blood urea, haemogram, eritrosedimentation rate, hepatogram, ionogram, c3 and c4 components of the complement, creatinin and urine analysis. A search for toxic metals (Ta, Ar. Pb, Al and Hg) yielded negative results.

A brain CT scan gave normal results and the MRI of the cervical spine showed posteromedial disk herniation at c3-c4 and c4-c5 levels, but without spinal cord compression. A lumbosacral CT scan disclosed L5 degenerative disk disease without roots involvement.

The EMG studies demonstrated fibrillations, positive sharp waves and fasciculations in thenar muscles and first dorsal interosseus of the left hand and in the left tibialis anterior muscle. On maximal voluntary effort a diminished interference pattern was found in muscles of the upper and lower limbs, in both masseters and in the tongue; the loss of functional motor units was more pronounced in the lower limbs, mainly in the left tibialis anterior muscle. Most of the remaining motor unit potentials were fragmented or poliphasic, some of them had enlarged amplitudes. Nerve conduction testing showed normal motor conduction in the left ulnar (55 m/s), left median (52 m/s), right ulnar (60 m/s) and right median (55 m/s) nerves. Stimulation of the left deep peroneal nerve did not evoke extensor digitorum brevis muscle response, while the stimulation of the right deep peroneal nerve yielded a rather prolonged latency of 8.6 ms to the tibialis anterior muscle. F waves studies in the right upper limbs gave latency values of 31 and 30 ms (controls < 28 ms) when performed in the median and ulnar nerves respectively by stimulating the nerve trunks at the wrist, suggesting demyelination of motor fibers at their most proximal segments. No conduction block was observed in any of the explored nerves. Sensory nerve action potentials could not be detected either in the median or sural nerves.

A sural left biopsy was carried out; a fragment of the nerve was frezzed in isopentane cooled in liquid nitrogen for immunohistological studies. The other fragment was fixed for 20 minutes in glutaraldehyde 2.5% in phosphate buffer at 4ªC and, then, it was cut in several fragments for different procedures. Two nerve segments were post-fixed in 10% formalin, embedded in paraffin and transverse and longitudinal sections were stained with hematoxylin eosin, PAS-Schiff, Congo red and Zielh-Nielsen. One fragment was fixed in glutaraldehyde for 24 hs, embedded in Epon 812 and semithin sections were stained with toluidine blue. Two short and one long segments were post-fixed in 2% osmium tetroxide during 24 hs at 4ºC; the short fragments were embedded in paraffin; transverse and longitudinal sections without counterstain were used to evaluate the myelin fibers. The long fragment was immersed in glycerin for 72 hs and used to tease the myelinated fibers.

The histoimmunological studies were made on transverse criostat tissue sections fixed in cooled acetone. «In situ» deposits of IgG and IgM were investigated in the tissue sections by the direct immunofluorescent technique incubating with fluorescein-conjugated rabbit anti-human IgG and IgM specific antisera (Dako Corp), diluted 1/10 in PBS, during 60 minutes at room temperature. «Circulating» IgG and IgM antibodies directed to nerves structures wre investigated in the same manner but a preincubation was conducted on the tissue sections with the patient serum diluted 1/10 and 1/40 in PBS for 60 minutes at room temperature.

The routine techniques revealed no abnormalities in the epineurium and perineurium; slight thickening of the blood vessel walls was observed. The slides of osmium post-fixations for myelin fibers and the semithin sections showed mild loss of mainly thick myelinated fibers. Isolated degenerating fibers, signs of remyelination and ax-

onal regeneration were also found. The teasing of single myelin fibers demonstrated the existence of some axonal degeneration, segmental and paranodal demyelination and remyelination.

The immunofluorescence studies for detection of IgG revealed an increment of the normal intensity of positive reaction in the endoneural space plus abnormal positivity of the myelina fibers. This stain pattern was increased in the slides previously incubated with the patient serum either at 1/10 or 1/40 dilution.

The investigation of IgM showed slight subperineurial and perivascular positive immunoreactivity and marked fluorescein staining of the myelin fibers; the endoneurial space showed no strong reaction. It was not possible to establish accurately the exact structure stained by the immunofluorescence technique. Increase was found in the intensity of staining with previous incubation of the tissue sections with the patient serum.

The patient was put on cyclophosphamide, 1.5 g as the initial dose, administered by vein puncture, and repeated a month later. Thereafter, the patient received 100 mg daily by oral route. The blood white cells count was 4000, or a bit less, on repeated blood samples throughout the therapeutical period.

Four months after the starting of the treatment, his strength slightly improved, mainly in the lower limbs. A faint movement appeared on both feet, MRC values for both peroneal muscle groups were 2/5 and for both soleus and gastrocnemi muscles 3/5. Muscle bulks slightly enlarged and fasciculations became less obvious. His gait and posture also improved. Tendon jerks were unchanged. Serum levels of antibodies anti-GM1 deminished (1/3200) and sensory conduction reappeared in the left median nerve with a speed value slightly diminished (45 m/s) and a sensory action potential amplitude of 10 uV; sural nerve action potential could not be detected on the non-operated side.

The immunofluorescence studies were done again on the remaining sural nerve 8 months after the starting of the treatment and, at this time, no abnormal reactivity was observed, even after incubation the nerve with undiluted serum of the patient.

Patient 2

A 74-year-old man (H.G.) developed cramps and progressive weakness of the lower limbs beginning one year before he was admitted into the hospital.

There was no family history of any neurological disease.

In the neurological clinical examination he was an alert and pleasant man. Some of his muscles were weak; MRC scale disclosed these values: 4/5 for the left deltoid and biceps, 4/5 for the wrist extensors and flexors of both upper limbs, 4/5 for the interosseus muscles of both hands, 0/5 for the peroneal group of the right leg, 2/5 for the same muscle group of the left leg, with consequent foot-drop on both sides, and 4/5 for the plantar flexion of both big-toes. The other values were normal. Mild muscle wasting was observed in left deltoid and biceps, both thenar and hypothenar groups and left peroneal muscles, severe wasting was found in the right peroneal muscles group. Achilles tendon jerks were absent bilaterally, while the knee jerks were diminished and the upper limbs jerks were slightly brisk. Fasciculations could be found in proximal and distal muscles of the four limbs.

Sensation was normal in the upper limbs, but impaired in the lower limbs where distal hypalgesia and diminished vibration sense were found on both sides.

The remainder of the neurological and general clinical examination was normal.

Laboratory testing showed increased titers of serum anti-GM1 antibodies (patient: > 1/1600, controls < 1/800) (ELISA).

A search for toxic metals (Ta, Ar, Pb, Al, Hg) yielded negative results. On serum immunoelectrophoresis quantitative IgM, IgG and IgA immunoglobulins were within the normal range, without a detectable paraprotein. Hemogram, blood urea and glucose, eritrosedimentation rate, hepatogram, creatinin and urine analysis were also normal.

A MRI of the cervical spine disclosed multiple disks degeneration slightly narrowing the spinal channel, but sparing the spinal cord.

EMG recordings showed fibrillations, positive sharp waves and fasciculations in proximal and distal muscles of the four limbs. On voluntary muscle contraction a reduced interference pattern was found in all limbs and cranial muscles. The interference pattern was poorer in distal muscles of the lower limbs, mainly in the right peroneal group where no voluntary activity could be detected. Most of the remaining motor unit potentials were fragmented or poliphasic and had enlarged amplitudes.

Motor conduction velocities yielded normal values for the right median (56 m/s), left median (62 m/s), right ulnar (61 m/s), left deep peroneal (42 m/s), right posterior tibial (47 m/s) and left posterior tibial (48 m/s) nerves, while a slightly reduced value was obtained in the left ulnar nerve (47 m/s), suggesting mild demyelination, and no muscle response could be detected when stimulating the right deep peroneal nerve. The F waves studies conducted on the right ulnar nerves gave values of 26 ms (controls: < 28 ms). No conduction block was noted in any nerve. Sensory action potentials could not be obtained either in the median or sural nerves.

The sural nerve biopsy was processed with the same methods and techniques as in the first patient. No abnormalities in the epineurium, perineurium and blood vessels were found. The endoneurial nuclei and collagen density

was normal and mild diminution of the myelin fibers density was observed. Isolated degenerating fibers, signs of remyelination and axonic clusters signaling axonal regeneration were found. Axonal degeneration, segmental and paranodal demyelination and remyelination were seen in the teased of single myelin fibers preparations. The immunofluorescence studies demonstrated abnormal reactivity for IgG in the myelin fibers which increased after incubating the nerve sample with the patient's serum.

Discussion

Increased titers of anti-GM1 antibodies were found in subpopulations of patients with lower motor neuron disease, motor neuropathy with or without motor conduction block, chronic sensorimotor neuropathy and Guillain-Barre syndrome[1, 2, 3]. These antibodies are clinically nonspecific and are not associated with any particular clinical syndrome. However, it is acknowledged that they are closely related to a clinical syndrome that is purely or predominantly motor and resembles motor neuron disease in most cases. These patients have simmetric or asymmetric weakness, cramps, and fasciculations, with active or absent deep tendon reflexes but without definitive, in most cases, upper motor neuron signs[7, 8, 9]. Currently, the meaning of the presence of anti-GM1 antibodies in patients with such peripheral nervous system diseases are controversial; some authors suggested that the antibodies have a major role in their development, while others denied their role in the pathogenesis of those illnesses.

Most of the reported cases in the literature have related the presence of anti-GM1 antibodies to pure motor peripheral involvement and seldom the peripheral sensory system has been found affected[8]. As far as we know, the first report which mentions the binding of IgM anti-GM1 antibodies to the human sural nerve is the one of Santoro et al.[4] who found this association in a patient with lower motoneurone signs, multifocal conduction block and doubtful signs of upper motoneurone involvement; they could localize the binding at the nodes of Ranvier within the sural nerve fibers.

In our patients, the sensory involvement was electrophysiological and anatomical confirmed in both. However, the most interesting observation was the presence of IgG in both and IgM, in the first case, into the sensory fibres within the sural nerves, which became more conspicuous when the nerves were incubated with the patients sera.

We cannot be sure that the anti-GM1 antibodies play a role in the pathogenesis of the disease described in our patients or are only an associated abnormality, because we could not individualize neither the antibodies carried by the immunoglobulins nor the fine structure of the nerve fibers where the immunoglobulin attached. However, it is known that gangliosides are present at motor neurones somae and their processes and, also, at the nodes of Ranvier, mainly at the paranodal myelin[6]. Therefore, on theoretical grounds, immune reactivity to GM1 may cause damage of the motoneurone somae, axonal degeneration and demyelination. The clinical and electrophysiological signs disclosed in our patients showed that all these features were present in both of them. Besides, clinical features in the second case, and electrophysiological and anatomical evidences in both, also showed that the sensory fibers were incriminated as well.

Furthermore, in case 1 treatment with cyclophosphamide diminished the amount of circulating anti-GM1 antibodies yielding clinical and electrophysiological improvement and also disappearance of IgG and IgM serum immunoproteins within the sural nerve, strongly suggesting that immune mechanisms were involved in the pathogenesis of his disease.

In any case, it seems that the presence of anti-GM1 antibodies might be a marker signaling a potentially treatable immune disorder which may have signs of lower and upper motor neurone diseases, albeit these last ones may be very mild, and also clinical and electrophysiological evidences of peripheral sensory involvement.

These observations have extended a bit more the increasing knowledge of these still ill-defined pahologies showing that peripheral sensory fibers may participate of the damage and may produce clinical and electrophysiological signs readily recognizable. On the other hand, also it has been shown that immunosuppressive therapy can be a powerful tool which benefits some of these patients.

Resumen

Síndrome de neurona motora inferior asociado a anticuerpos anti-GM1

Recientemente se ha reconocido la existencia de enfermedades de motoneurona, con o sin bloqueo de conducción nerviosa periférica, de neuropatías sensitivo-motoras y del síndrome de Guillain Barré asociados a títulos séricos elevados de anticuerpos anti-GM1. Santoro y col[4]. fueron los primeros en demostrar que los anticuerpos anti-GM1 eran capaces de depositarse en los nodos de Ranvier del nervio sural de un paciente con un cuadro clínico que recordaba a una esclerosis lateral amiotrófica y que poseía altos títulos de anticuerpos anti-GM1.

Los pacientes presentados en este trabajo tenían títulos elevados de anticuerpos anti-GM1 en el suero y expresión clínica de enfermedad de motoneurona asimétrica, con signos y síntomas de compromiso de neurona motora inferior en ambos casos y con signos sugestivos, en el primer paciente, de lesión de neurona motora superior y, en el segundo, de alteración sensitiva en ambos miembros inferiores. Electrofisiológicamente no se evidenció bloqueo de conducción motora en ninguno de ellos, pero sí se observó la inexcitabilidad de las fibras sensitivas de los nervios mediano y sural en ambos. En los dos enfermos la biopsia del nervio sural mostró la presencia de inmunoglobulinas dentro de las fibras nerviosas.

Los hallazgos hechos señalan que la presencia de títulos positivos de anticuerpos anti-GM1 en el suero parecería ser un marcador de un tipo de enfermedad de neurona motora, potencialmente tratable, en la que es posible encontrar signos combinados de compromiso de primera y segunda neuronas, y que, además, puede cursar con alteración sensitiva demostrable clínica y electrofisiológicamente.

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LA PORTADA

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