Anti-GM1 and anti-sulfatide antibodies in patients with systemic lupus erythematosus, Sjögren's syndrome, mixed cryoglobulinemia and idiopathic systemic vasculitis

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Abtract

Objectives

Over the last two decades, increasing interest has been focused on the association between autoimmune polyneuropathies and anti-neuronal autoantibodies in immune-mediated polyneuropathy. The possible appearance of these autoantibodies in systemic diseases that are not limited to the nervous system has not been fully addressed yet.

Methods

We evaluated 32 patients with systemic lupus erythematosus, 34 patients with hepatitis C virus-associated mixed IgM-k/IgG cryoglobulinemia, 19 with small vessel ANCA-associated vasculitis, and 20 patients with Sjögren's syndrome by means of an immunoenzyme method of anti-neuronal autoantibody detection.

Results

As compared to normals, a significant increase ($p < 0.001$) in plasma titers of both IgM and IgG anti-GM1 ganglioside and IgM and IgG anti-sulfatide was observed in patients with systemic lupus erythematosus, mixed cryoglobulinemia and Sjögren's syndrome. Idiopathic systemic vasculitis patients were found to have significantly increased levels of anti-sulfatide IgG autoantibodies ($p < 0.001$). Clinical and electrophysiologic studies revealed that abnormal titers of anti-neuronal antibodies were associated with evidence of neuropathy in patients with systemic lupus erythematosus and ANCA-related vasculitis ($p < 0.05$) as well as in patients with mixed cryoglobulinemia and Sjögren's syndrome ($p < 0.001$).

Conclusion

Anti-GM1 and anti-sulfatide antibodies are frequently found in patients with small vessel ANCA-associated vasculitis and other multi-organ immune-mediated diseases. Upon detection of these antibodies, accurate neurologic examination should be carried out due to the significant association that can be found between these serologic abnormalities and the involvement of the peripheral nervous system as also detected by electrophysiologic studies. This study supports the unexpected possibility that anti-neuronal reactivity may be a direct trigger of neurologic injury in these systemic disorders.

Key words

Anti-neuronal antibodies, systemic lupus erythematosus, mixed cryoglobulinemia, ANCA-associated vasculitis, Sjögren's syndrome.

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Introduction

Over the last two decades, increasing interest has been focused on the association between autoimmune polyneuropathies and high titers of anti-neuronal antibodies, *i.e.*, antibodies anti-GM1 ganglioside and anti-sulfatide (1-5).

The mechanism by which these antibodies might cause nerve injury remains unclear (6-8). Low to moderate titers of both anti-ganglioside and anti-sulfatide autoantibodies can be found in non-immune-mediated polyneuropathies, and low affinity anti-neuronal antibodies are part of the normal human immunological repertoire (5, 9, 10).

Polyneuropathies are frequently observed in systemic idiopathic vasculitis (VAS), such as ANCA-associated angiitis, or in others multi-organ immune-mediated diseases such as systemic lupus erythematosus (SLE), mixed cryoglobulinemia (MC), and Sjögren's syndrome (SS), which are characterized by a broad spectrum of clinical manifestations and several immunologic abnormalities. Nevertheless, peripheral nervous system involvement has not been extensively investigated by an electrophysiologic approach, thus the reported prevalence of peripheral neuropathy in these conditions varies among studies, and reflects the different criteria that are used to clinically define polyneuropathies (11-14).

The occurence of autoimmune diseases (*i.e.*, SLE) associated with *a priori* idiopathic polyneuropathy has previously been reported (15). Furthermore, serum anti-neuronal antibodies have been detected in SLE (16-19) and primary Sjögren's syndrome (20-22).

In the present study, anti-ganglioside GM1 and anti-sulfatide antibodies were detected in several patients with idiopathic systemic vasculitis and hepatitis C virus (HCV)-associated MC, as well as SLE and SS. The results were related to some serologic parameters of disease activity and electrophysiologic indices of peripheral neuropathy.

Methods

Patients

Serum samples were obtained from 32 patients with systemic lupus erythematosus, 34 patients with HCV-associated mixed (IgM-k/IgG) cryoglobulinemia, 19 patients with small vessel ANCA-associated idiopathic vasculitis, and 20 patients with Sjögren's syndrome. Both patients symptomatic for peripheral neuropathy and asymptomatic patients were considered elegible for the study. Each patient gave informed consent.

Diagnosis of SLE was established according to the 1982 American Rheumatism Association revised criteria (23). Demographic data are shown in Table I. Clinical activity was established by assessing SLEDAI score system. Diagnosis of hepatitis C virus-associated MC and SS were based on previously established criteria (24, 25), while diagnosis of small vessel ANCA-associated vasculitis was made according to Jennette *et al*. (26). Sixteen patients with SLE, 10 with MC, 10 with ANCA-associated idiopathic vasculitis and 5 with SS were in mild activity or in clinical and serological remission at the time of study. Sera of 34 healthy blood donors and reference sera from antibody positive patients with Guillain Barrè syndrome were used as negative and positive controls, respectively (27). The diagnosis of neuropathy was made in the presence of at least one of the following criteria: weakness, decrease or loss of tendon reflexes, sensory symptoms such as paresthesia or dysesthesia. Neuropathy was assumed to be secondary to SLE, SS, MC or idiopathic systemic vasculitis if all of the following features were absent: history or laboratory findings suggestive of diabetes mellitus, hypothyroidism, uremia, vitamin B1, B12 or E deficiency, alcohol abuse, HIV-infection or Lyme's disease, trauma, paraneoplastic syndromes, hereditary neuropathies. Based on clinical examination, patients were grouped into three categories: sensory neuropathy, motor neuropathy and mixed sensory-motor neuropathy (28).

Antibody assay

Sera from all patients and controls were tested by an enzyme-linked immunosorbent assay (ELISA) for the presence of IgM-IgG antibodies to GM1 gangliosides and sulfatides. The ELISA test was carried out by adding 500 ng of either purified GM1 (Sigma,

Table I. Demographic characteristics of the patients under study.

	SLE	MC	VAS	SS
Total	32	34	19	20
Age, years (mean ± SD)	42 ± 17	61 ± 12	61 ± 15	64 ± 10
Gender: Female [n (%)]	28 (87%)	19 (56%)	7 (37%)	18 (90%)
Male	4 (13%)	15 (44%)	12 (63%)	2 (10%)
Disease duration: years (range)	9 (4-14)	8 (3-13)	9 (4-14)	5 (2-8)
Pts treated with Steroids and/or Immunosuppressive drugs	23	20	17	6

SLE: systemic lupus erythematosus; MC: mixed cryoglobulinemia; VAS: idiopathic systemic vasculitis; SS: Sjögren's syndrome.

St. Louis, MO, USA) or sulfatide (Sigma, St. Louis, MO, USA) in 100 µl of methanol to each well of a microtiter plate (Pharmacia & Upjohn, Michigan, USA). After methanol evaporation, wells were blocked by incubation with 200 µl 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) and incubated overnight at 4°C. Wells were then washed three times with 250 µl 1% BSA in PBS, and 100 µl aliquots of the patient's serum were serially diluted in washing buffer (starting with a dilution of 1:200 for anti-GM1 antibody and 1:800 for anti-sulfatide antibody) and then added in duplicate and incubated overnight at 4°C. After washing twice, 100 µl aliquots of peroxidase-conjugated rabbit anti-human IgM or IgG (Dako, Ontario, Canada) diluted at 1:1000 in buffer were added and incubated overnight at 4°C. After washing again, 100 µl of a developing solution containing 0.1% o-phenylenediamin (Sigma, St. Louis, MO, USA) in 0.1 M citric acid buffer and 0.04 % H_2O_2 were added, and the reaction was stopped by adding 100 µl 1 N sulphuric acid 20 minutes later. Lastly, the plates were incubated at room temperature for 30 min before measuring the absorbance at 490 nm with an ELISA counter. Antibody titers were calculated as previously described by Pestronk *et al*. (4). Reference sera from patients with Guillain-Barrè syndrome with high titers of IgM anti-GM1, or IgM anti-sulfatide antibodies were used to determine inter- and intra-assay variability. Accuracy data, as determined by Receiver Operator Characteristic analysis of anti-neuronal antibody levels applied in polyneuropathies, including Guillain Barrè syndrome, have been described elsewere (29).

Other laboratory parameters

Antinuclear antibodies (ANA) and antidsDNA were detected by immunofluorescence (IF) on HEP2 cells (Diasorin s.p.a., Vercelli, Italy) and immunofluorescent Chritidia test (Daltec Instrument s.r.l., Milan, Italy), respectively. ANA was also detected by EIA (Bouty s.p.a., Milan, Italy) and Rheumatoid factor was determined by immunoturbidimetric assay (Roche, Monza, Italy). Antineutrophil cytopasmic antibodies were detected by I.F. (Daltec Instrument s.r.l., Milan, Italy) and by EIA (Bouty s.p.a., Milan, Italy). Anti-Ro/SSA and anti-La/SSB antibodies were detected by ELISA (Bouty s.p.a., Milan, Italy) in 16 out of 20 SS patients. Cryoglobulins were determined as follows. After isolation and washing, the cryoprecipitate was quantified and expressed as a percentage of precipitate/serum volume (*i.e*., cryocrit). Components of the cryoprecipitate were characterized by using immunofixation electrophoresis (CRYOKIT, Helena Labs, Milan, Italy).

Electrophysiologic studies

A careful neurological examination could be done in 30 of 32 patients with SLE, 33 of 34 patients with cryoglobulinemia, 18 out of 20 patients with Sjögren's syndrome and all 19 with idiopathic systemic vasculitis at the time of blood sampling. Each neuropathy established on a clinical ground was electrophysiologically examined. The following electrophysiologic investigations were done on both legs in 12 out of 30 SLE, 22 out of 33 cryoglobulinemic patients, 8 out of 19 patients with ANCA-positive systemic vasculitis and 11 out of 18 patients with Sjögren's syndrome. Motor conduction velocities were determined on deep peroneal nerves. Interval between stimulating and recording electrodes was 8 cm at ankle. M wave amplitude, distal latency, and maximal conduction velocity were considered. Antidromic sensory velocities were done on sural nerve at external malleolus-surae tract. Sensory potential amplitude and maximal conduction velocity were measured. Neuropathies were classified as demyelinating when the neurophysiologic criteria for the demyelination, as defined by the "ad Hoc Subcommittee" (*i.e*., critical reduction of conduction velocity with normal M wave amplitude and increased distal latency), were fulfilled (30). In the presence of a decrease in M wave amplitude and temporal dispersion with < 30% reduction of conduction velocity, the neuropathy was classified as axonal. When criteria for both demyelinating and axonal neuropathy were fulfilled, a mixed form was diagnosed. Values for nerve conduction velocity and nerve action potentials were compared with control values obtained in 34 healthy, age-matched subjects. Patients' variables were considered abnormal if outside the standard deviation range.

Statistical analysis

Titers of anti-GM1, anti-sulfatide antibodies and electrophysiologic data among the groups were compared by Student's t-test (associated to Bonferroni's correction when necessary). The χ^2 and Fisher's Exact Test were used to determine the relationship between the presence of neuropathy and type of nerve damage (axonal, demyelinating, or axonal and demyelinating) and the detection of anti-GM1 and/or anti-sulfatide antibodies. The same analysis was used to determine the correlation between SLEDAI score and anti-neuronal antibodies. Pearson's correlation coefficients were used to determine possible associations between titers of anti-neuronal antibodies and antineutrophil cytoplasmic antibodies (ANCA), antidsDNA, ANA, anti-Ro/

SSA, anti-La/SSB, presence of rheumatoid factor (RF) and cryocrit. *P* values < 0.05 were considered statistically significant.

Results

A significant increase in plasma IgM and IgG anti-GM1 titers was found in the SLE patients (1:618 ± 1:514; 1:465 ± 1:308) as compared to normal controls (1:176 ± 1:205 and 1:204 ± 1:103; p < 0.001). IgM and IgG anti-sulfatide titers (1:2941 ± 1:2734; 1:1580 ± 1: 1166) were also found to be higher than in control subjects (1:1042 ± 1:840 and 1:621 ± 1:243; $p < 0.001$). Mean plasma IgM and IgG anti-GM1 antibody titers of cryoglobulinemic patients were 1:524 ± 1:384 and 1:488 ± 1:389, respectively, which again were significantly higher than normal controls ($p < 0.0001$). Plasma IgM and IgG anti-sulfatide titers in this group (1:1841 ± 1:1101 and 1:1200 ± 1:829) were also significantly higher than in normal subjects ($p < 0.001$). A significant increase in anti-sulfatide IgG class levels (1:1400 ± 1:708) was observed in the vasculitis subjects as compared to controls ($p < 0.001$). Mean plasma IgM and IgG anti-GM1 titers of patients with Sjögren's syndrome were respectively, 1:811 ± 1:334 and 1:538 ± 1:247, again higher than in controls ($p < 0.001$). Mean plasma IgM and IgG anti-sulfatide titers in this group were 1:2079 ± 1:948 and 1:1890 ± 1:483 ($p < 0.001$ as compared to normal controls). These results are summarized in Figures 1 and 2.

Clinical neurological examination was found to be abnormal in 12 out of 30 SLE patients (40%), in 22 of the 33 cryoglobulinemic patients we studied (67%), in 8 of the 19 ANCA-related vasculitis patients (42%), and in 11 out of the 18 patients with Sjögren's syndrome who were tested (61%).

Table II summarises the clinical and electrophysiological findings. In 13 patients (5 with SLE, 5 with cryoglobulinemia, 2 with Sjögren's syndrome and 1 with ANCA-positive systemic vasculitis patients) a predominantly sensory neuropathy was found. In 31 patients (4 with SLE, 16 with cryoglobulinemia, 6 with Sjögren's syndrome and 5 with ANCA-positive systemic vasculitis) a mixed sensory and motor neuropathy was detected. The remnant patients (3 with SLE, 1 with cryoglobulinemia, 3 with Sjögren's syndrome and 2 with ANCA-positive systemic vasculitis patients) had prevalent signs of motor neuropathy.

Examination of the neurophysiological parameters showed some prevalence of mixed features of axonal and demyelinating neuropathy in MC and VAS neuropathic patients (15 out of 22 and 7 out of 8, respectively). Mixed and axonal forms of neuropathy were equally observed in SLE and SS patients.

A significant difference was found between patients with or without clinical signs of neuropathy as regard to anti-neuronal antibodies (Table III).

With regards to sensitivity of antibody detection in patients with electrophysiologic abnormalities, 11 of the 12 (91%) SLE patients with abnormal electrophysiologic data were found to have anti-neuronal (anti-GM1 and/or anti-sulfatide) antibodies. Abnormal titers of anti-neuronal antibodies were found to be associated with electrophysiologic abnormalities in 19 out of 22 (86%) cryoglobulinemic patients, 5 out of 8 (62%) ANCA-related vasculitis patients, and in 10 out of 11 patients (91%) with Sjögren's syndrome. Specificity for neuronal involvement of antibody detection was 61% (11 antibody-negative patients out of 18 patients without electrophysiologic abnormalities) in SLE, 82% (9 out of 11 patients) in MC, 100% (11 out of 11 patients) in idiopathic systemic vascu-

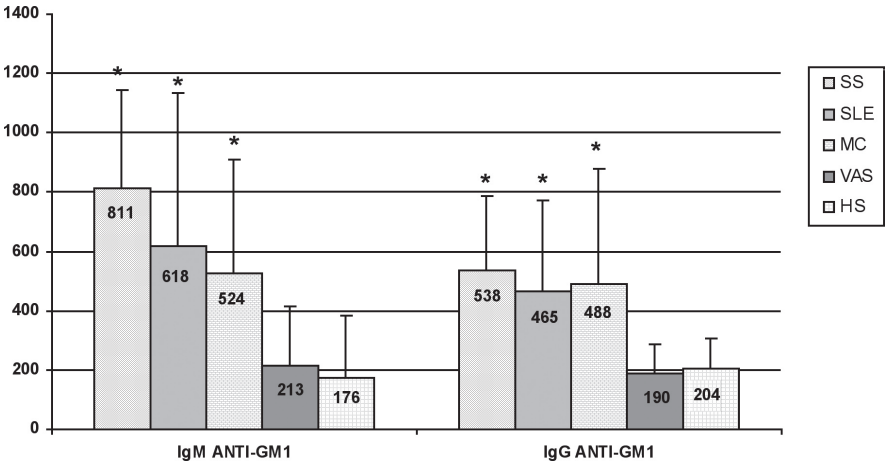

Fig. 1. IgM and IgG anti-GM1 titers expressed as mean ± SD in Sjögren's syndrome (SS), SLE patients (SLE), Mixed Cryoglobulinemia (MC), idiopathic systemic vasculitis (VAS) patients, and Healthy Subjects (HS). $^{*}p < 0.001$ as compared to HS.

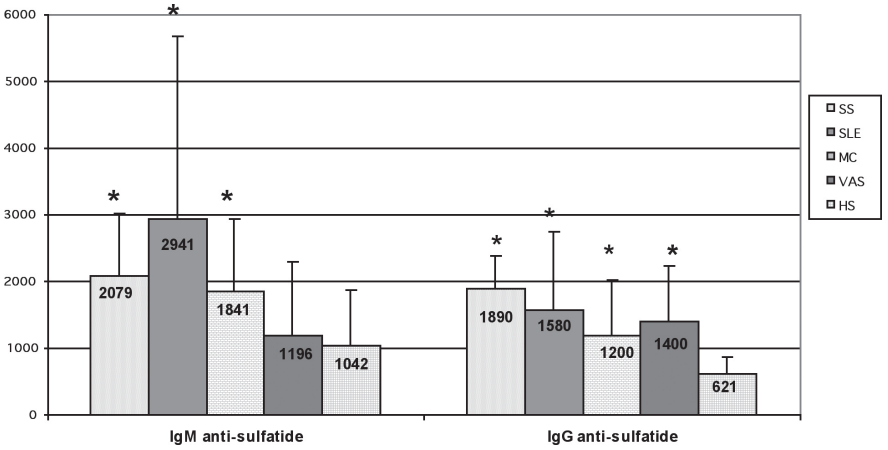

Fig. 2. IgM and IgG anti-sulfatide titers expressed as mean ± SD in Sjögren's syndrome (SS), SLE patients (SLE), patients with Mixed Cryoglobulinemia (MC), in idiopathic systemic vasculitis (VAS) patients and Healthy Subjects (HS). $^{*}p < 0.001$ as compared to HS.

Table II. Summary of abnormal neurological findings in patients who had undergone a complete clinical evaluation (upper pannel), including electrophysiologic data in patients with abnormal neurologic examination. Six MC patients, 1 VAS patient and 3 SS patients had nonmeasurable amplitude values (assumed to be equal to zero).

		SLE (#30)	MC (#33)	VAS (#19)	SS (#18)
Sensory, n (%)		5 (17%)	5 (15%)	1 (5%)	2 (11%)
Motor, n (%)		3 (10%)	1 (3%)	2 (11%)	3 (17%)
Sensory/motor, n (%)		4 (13%)	16 (49%)	5 (26%)	6 (33%)
Total, n (%)		12 (40%)	22 (67%)	8 (42%)	11 (61%)
	HS (#34)	SLE (#12)	MC (#22)	VAS (#8)	SS (#11)
Axonal Neuropathy					
Low MAP and/or SAP, n (%)		7 (23%)	2 (6%)	1 (5%)	7 (39%)
Demyelinating Neuropathy					
Slow MCV and/or SCV, n (%)		0	5 (15%)	0	0
Mixed form, n (%) Sural		5 (17%)	15 (46%)	7 (37%)	4 (22%)
Peroneal DL (sec)	3.8 (0.6)	3.7 (0.7)	4.8 (1.6)*	4.4 (0.9)	4.8 (1.8)**
Peroneal MCV (m/SEC)	49.1 (4.4)	46.5 (5.4)	37.2 (15.1)*	40.1 (9.6)**	43.2 (3.7)*
Peroneal AMP (mV)	6.3 (2.4)	5.7 (2.7)	2.2 (1.6)*	4.1 (3.7)	1.8 (1.3)*
Sural SAP (μV)	15.5 (6.7)	7.2 (3.4)*	3.9 (4.6)*	5.5 (3.4)*	4.5 (4.0)*
Sural SCV (m/SEC)	48.0 (3.0)	46.0 (6.3)	23.0 (21.0)*	34.1 (21.0)**	30.7 (23.2)*

MAP: motor action potential amplitude; SAP: sensory action potential; DL: distal latency; MCV: motor conduction velocity; SCV: sensory conduction velocity; HS: Healthy Subjects; SLE: SLE patients; MC: Mixed Cryoglobulinemia; VAS: ANCA-related vasculitis patients and SS: Sjögren's syndrome patients. Neuronographic findings are expressed as mean (SD). $^{*}p < 0.01$; $^{**}p < 0.05$ as compared to HS.

litis, and 88% (7 of 8 patients) in Sjögren's syndrome. Significant associations between the presence or absence of polyneuropathy and anti-neuronal antibodies were determined by the χ^2 test (Fig. 3).

The relationship between type of nerve damage and presence of anti-neuronal antibodies in patients with SLE, cryoglobulinemia, Sjögren's syndrome and idiopathic systemic vasculitis is shown in Table III. MC patients with anti-neuronal antibodies were found to have more often a mixed axonal and demyelinating neuropathy, while antibody-positive SS patients more frequently had an axonal damage.

No statistical correlation was found between anti-neuronal antibodies and some serologic markers of disease activity, including ANA in SLE, ANCA titers in idiopathic systemic vasculitis, RF or cryocrit in MC, anti-Ro/SSA and anti-La/SSB in SS (Table IV), nor with clinical and histologic extent of renal involvement. A weak correlation was observed between IgG anti-sulfatide and anti-DNA antibodies ($p = 0.05$).

SLEDAI score was > 15 in 5 SLE patients, > 10 and ≤ 15 in 6, > 5 and ≤ 10 in 13, ≤ 5 in the remaining 8. Some relationship ($p < 0.05$) was found between SLEDAI score and IgG anti-sulfatide antibodies by dividing patients in 2 subsets with > 10 or ≤ 10 SLEDAI score.

Table III. Relationship between type of nerve damage and presence of anti-neuronal antibodies. The number of patients with elevated anti-neuronal antibodies and neuropathy and the percentage value as referred to the entire patient sample is shown in the last column. One systemic lupus eythematosus (SLE), 3 mixed cryoglobulinemia (MC), 3 idiopathic systemic vasculitis (VAS) and 1 Sjögren's syndrome (SS) neuropathic patients were antibody-negative. $^{*}p < 0.01$ and $^{**}p < 0.05$ as compared to antibody-positive patients without neuropathy.

	Axonal	Demyelinating	Mixed	
SLE	6 (20%)	0 (0%)	5 (17%)	11 (37%)
MC	2 (6%)	4 (12%)	13 (39%)	19 (57%)
VAS	1 (5%)	0 (0%)	4 (21%)	5 (26%)
SS	7 (39%)	0 (0%)	3 (17%)	10 (55%)

Discussion

The reported prevalence of neuropathy in primary systemic vasculitis, SLE, mixed cryoglobulinemia and SS is possibly underestimated (31-33). The present study showed that approximately 60% of the patients with SLE (20 out of 32) or mixed cryoglobulinemia (21 of 34), 55% of the patients with Sjögren's syndrome (11 out of 20) and 26% of patients with small vessel ANCA-associated idiopathic systemic vasculitis (5 out of 19) had increased levels of anti-ganglioside and/or anti-sulfatide antibodies. These patients were often found to have electrophysiologic abnormalities of the peripheral nervous system and, conversely, most of the patients with electrophysiologic abnormalities also had increased levels of anti-neuronal antibodies.

Peripheral neuropathy is generally thought to be caused by small vessel inflammatory vasculopathy of the vasa nervorum. As recently emphasized, the blood-nerve barrier is not as efficient as the blood-brain barrier at limiting the inflammatory cell infiltration into the vasa nervorum and epineural arteries, and is susceptible to ischemic injuries and prone to develop blood-nerve barrier breaches due to release of reactive oxigen species, cytokines and metalloproteinases (34).

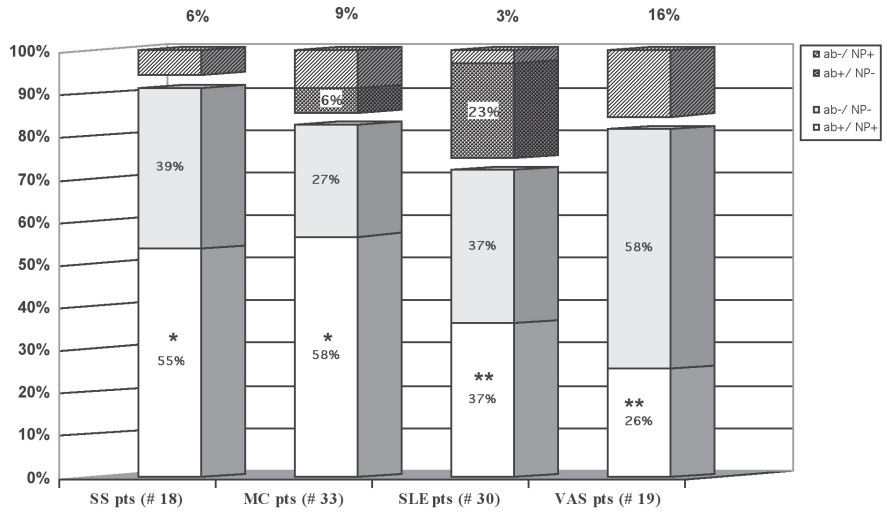

Fig. 3. Percentage of patients with (ab+) or without (ab-) antineuronal antibodies related to the presence (NP+) or absence (NP-) of neuropathy. $^{*}p < 0.001$ and $^{**}p < 0.05$ by the χ^2 test.

Table IV. Serologic markers of disease activity (expressed in mean ± SD) and anti-GM1 and anti-sulfatide antibodies in SLE patients, patients with mixed cryoglobulinaemia (MC), patients with ANCA-associated vasculitis (VAS) and in SS patients. Pearson's correlation coefficients are shown.

Serum markers	IgM anti-GM1	IgG anti-GM1	IgM anti-sulfatide	IgG anti-sulfatide
ANA				
in SLE pts (#32)	R^2: -0.03	R^2: -0.03	R^2: 0.03	R^2: -0.031
1.7 ± 1 AU	(p= 0.82)	(p = 0.70)	(p = 0.16)	(p = 0.81)
(n.v. < 0.7)				
anti-dsDNA				
in SLE pts (#32)	R^2: 0.033	R^2: -0.03	R^2: 0.08	R^2: 0.1
99 ± 131 IU/ml	(p = 0.15)	(p = 0.76)	(p = 0.06)	(p = 0.05)
(n.v. < 30)				
RF				
in MC pts (#34)	R^2: -0.01	R^2: -0.01	R^2: 0.005	R^2: 0.011
525 ± 1880 IU/ml	(p = 0.44)	(p = 0.42)	(p = 0.29)	(p = 0.25)
(n.v. < 14)				
ANCA				
in VAS pts (#19)	R^2: 0.10	R^2:-0.05	R^2: 0.138	R^2: -0.05
1.5 ± 1.3 IU/ml	(p = 0.09)	(p = 0.84)	(p = 0.06)	(p = 0.8)
(n.v. < 2)				
anti-Ro/SSA; anti-La/SSB				
in SS pts (#16)	R^2:0.013	R^2:-0.06	R^2:0.10	R^2:-0.040
39 ± 51 IU/ml	(p = 0.29)	(p = 0.74)	(p = 0.11)	(p = 0.5)
(n.v. < 10 IU/ml)				
RF				
in SS pts (#20)	R^2:-0.05	R^2:-0.022	R^2:-0.05	R^2:-0.03
29 ± 20 IU/ml	(p = 0.93)	(p = 0.45)	(p = 0.86)	(p = 0.51)

Anti-ganglioside and anti-sulfatide antibodies might be the result of a phlogistic and ischemic damage which exposes normally segregated neuronal epitopes. Their presence in serum might reflect a secondary immune reaction to neural damage.

Alternatively, the appearance of these autoantibodies may be the sequaela of a co-infection with bacteria or viruses that bear cross-reactive lipopolysaccharides, as demonstrated in the Guillain-Barré syndrome (35-37). Finally, anti-neuronal immune response might represent an independent pathway of the autoimmune repertoire of these multiorgan disorders, *i.e*, the expression of the polyclonal activation of B lymphocytes, a common feature of autoimmune diseases.

The levels of anti-GM1 and anti-sulfatide antibodies did not correlate with the values of ANA in SLE, or with ANCA titers in idiopathic systemic vasculitis, or with RF or cryocrit in cryoglobulinemia. A weak correlation was found between IgG anti-sulfatide and anti-DNA antibodies ($p = 0.05$). No data are presently available to determine whether IgM or IgG isotypes have different clinical impact as in other biological setting, such as antidsDNA or anticardiolipin antibodies. IgG might be more promising. For instance, IgM anti-sulfatide titers did not reach statistical levels of significance in the VAS group while IgG isotype did and IgG anti-sulfatide was found to be related to both antidsDNA levels and SLEDAI score.

While the present findings should be interpreted with caution due to the lack of significant correlations with other indices of disease activity, detecting anti-neuronal antibodies might provide additional diagnostic and prognostic insight into systemic immune-mediated disorders. Data on sensitivity and specificity showed patients with anti-neuronal antibodies to have definite damage of peripheral nervous system.

These antibodies might play a role in the pathogenesis of the neurological manifestations that are observed in ANCA-associated systemic vasculitis and in other multi-organ immune-mediated disease, such as Systemic Lupus Erythematosus, HCV-related mixed cryoglobulinemia and Sjögren's syndrome.

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References

1. NOBILE ORAZIO E, CARPO M, LEGNAME G, MEUCCI N, SONNINO S, SCARLATTO G: Anti-GM1 IgM antibodies in motor neuron disease and neuropathies. *Neurology* 1990; 40: 1747-50.
2. PESTRONK A: Motor neuropathies, motor neuron disorders and anti-glycolipid antibodies. *Muscle Nerve* 1991; 14: 927-36.

3. SADIQ SA, THOMAS FP, KILIDIREAS K *et al*.: The spectrum of neurologic diseases associated with anti-GM1 antibodies. *Neurology* 1990; 40: 1067-72.
4. PESTRONK A, LI F, GRIFFIN J, FELDMAN EL *et al*.: Polyneuropathy syndromes associated with serum antibodies to sulfatide and myelin-associated glycoprotein. *Neurology* 1991; 41: 357-62.
5. DABBY R, WEIMER LH, HAYS HP, OLARTE M, LATOV N: Anti-sulfatide antibodies in neuropathy. Clinical and electrophysiologic correlate. *Neurology* 2000; 54: 1448-52.
6. ARASAKI K, KUSONIKI S, KUDO N, KANASAWA I: Acute conduction block *in vitro* following exposure to anti-ganglioside sera. *Muscle Nerve* 1993; 16: 587-93.
7. TAKIGAWA T, YASUDA H, KIKKAWA R, SHIGETA Y, SAIDA T, KITASATO H: Antibodies against GM1 ganglioside affect K+ and Na+ currents in isolated rat myelinated nerve fibers. *Ann Neurol* 1995; 37: 436-42.
8. WEBER F, RÜDEL R, AULKEMEYER P, BRINKMEIER H: Anti-GM1 antibodies can block neuronal voltage-gated sodium channels. *Muscle Nerve* 2000; 23: 1414-20.
9. MC COMBE PA, WILSON R, PRENTICE LR: Results of testing for anti-GM1 antibodies. *J Clin Neurosci* 2000; 7: 209-12.
10. CARPO M, ALLARIA S, SCARLATO G, NOBILE ORAZIO E: Marginally improved detection of GM1 antibodies by Covalink ELISA in multifocal motor neuropathy. *Neurology* 1999; 53: 2206.
11. ESTES D, CHRISTIAN CL: The natural history of systemic lupus erythematosus by prospective analysis. *Medicine* 1971; 50: 85-95.
12. DUBOIS EL, TUFFANELLI DL: Clinical manifestations of systemic lupus erythematosus: computer analysis of 520 cases. *JAMA* 1964; 190: 104-11.
13. HUYNH C, HO SL, FONG KY, CHEUNG RTF, MOK CC, LAU CS: Peripheral neuropathy in systemic lupus erythematosus. *J Clin Neurophysiol* 1999; 16: 164-68.
14. ROALD O, SVEIN IM, LASSE G *et al*.: Small nerve fiber involvement in systemic lupus erythematosus. *Arthriis Rheum* 2002; 46: 1228-32.
15. VAN LAARHOVEN H W, ROOYER F A, VAN ENGELEN BG, VAN DALEN R, BERDEN JH: Guillain-Barré syndrome as presenting feature in a patient with lupus nephritis, with complete resolution after cyclophosphamide treatment. *Nephrol Dial Transplant* 2001; 16: 840-42.
16. GEORGESCU L, MEVORACH D, ARNETT FC, REVEILLE JD, ELKON KB: Anti-P antibodies and neuropsychiatric lupus erythematosus. *Ann N Y Acad Sci* 1997; 823: 263-69.
17. HASHIMOTO H: Peripheral neuropathies in lupus patients with anti-GM1 antibodies. *Intern Med* 1999; 38: 689-90.
18. GALEAZZI M, ANNUNZIATA P, SEBASTIANI GD *et al*.: Anti-ganglioside antibodies in a large cohort of European patients with systemic lupus erythematosus: clinical, serological, and HLA class II gene associations. European Concerted Action on the Immunogenetics of SLE. *J Rheumatol* 2000; 27: 135-41.
19. DEGIORGIO LA, KONSTANTINOV KN, LEE SC, HARDIN JA, VOLPE BT, DIAMOND BA: Subset of lupus anti-DNA antibodies cross-reacts with the NR2 glutamate receptor in systemic lupus erythematosus. *Nat Med* 2001; 7: 1189-93.
20. ZEBALLOS RS, FOX RI, CHERESH DA, MCPHERSON RA: Anti-glycosphingolipid autoantibodies in rheumatologic disorders. *J Clin Lab Anal* 1994; 8: 378-84.
21. BANSAL AS, ABDUL-KARIM B, MALIK RA *et al*.: IgM ganglioside GM1 antibodies in patients with autoimmune disease or neuropathy, and controls. *J Clin Pathol* 1994 Apr; 47: 300-02.
22. GIORDANO N, LUCANI B, AMENDOLA A *et al*.: IgG and IgM antiganglioside M1 antibodies in primary Sjögren's syndrome with and without peripheral neuropathy. *Clin Rheumatol* 2003; 22: 256-58.
23. TAN EM, COHEN AS, FRIES JF *et al*.: The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982; 25: 1271-77.
24. ROCCATELLO D, MORSICA G, PICCIOTTO G *et al*.: Impaired hepatosplenic elimination of circulating cryoglobulins in patients with essential mixed cryoglobulinemia and hepatitis C virus (HCV) infection. *Clin Exp Immunol* 1997; 110: 9-14.
25. VITALI C, BOMBARDIERI S, JONSSON R *et al*.: European Study Group on Classification Criteria for Sjögren's syndrome: Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis* 2002; 61: 554-58.
26. JENNETTE JC, FALK RJ, ANDRASSAY K *et al*.: Nomenclature of systemic vasculitides. Proposal of an international consensus conference. *Arthritis Rheum* 1994; 37: 187-92.
27. ALEADINI A, WIRGUIN I, LATOV N: Ganglioside agglutination immunoassay for rapid detection of autoantibodies in immune-mediated neuropathy. *J Clin Lab Anal* 2001; 15: 96-9.
28. THOMAS PK, OCHOA J: Clinical features and differential diagnosis. *In*: DYCK PJ, THOMAS PK, GRIFFIN JW, LOW PA, PODUSLO JF, eds. *Peripheral neuropathy.* 3rd ed. Philadelphia: W.B. Saunders, 1993: 749-74.
29. ISOARDO G, FERRERO B, BARBERO P *et al*.: Anti-GM1 and anti-sulfatide antibodies in polyneuropathies. Threshold titers and accuracy. *Acta Neurol Scand* 2001; 103: 180-87.
30. AD HOC SUBCOMMITTEE OF AMERICAN ACADEMY OF NEUROLOGY AIDS TASK FORCE: Research criteria for diagnosis of chronic inflammatory demyelinating polyneuropathy (CIPD). *Neurology* 1991; 41: 617-18.
31. COLLINS MP, KISSEL JT, MENDELL JR: Vasculitic neuropathies. *In*: ANTEL J, BIRNBAUM G, HARTUNG HP, eds. *Clinical Neuroimmunology*. Malden: Blackwell Science 1998; 316-39.
32. LHOTE F, GUILLEVIN L: Polyarteritis nodosa, microscopic polyangiitis, and Churg-Strauss syndrome: clinical aspects and treatment. *Rheum Dis Clin North Am* 1995; 21: 911-47.
33. DAVIES L, SPIES JM, POLLARD JD *et al*.: Vasculitis confined to peripheral nerves. *Brain* 1996; 119: 1441-48.
34. PAGNOUX C, GUILLEVIN L: Peripheral neuropathy in systemic vasculitides. *Curr Opin Rheumatol* 2005; 17: 41-8.
35. YUKI N, TAKI T, INAGAKI F *et al*.: A bacterium lipopolysaccharide that elicits Guillain-Barré syndrome has a GM1 ganglioside-like structure. *J Exp Med* 1993; 178: 1771-75.
36. PRENDERGAST MM, LASTOVICA AJ, MORAN AP: Lipopolysaccharides from Campylobacter Jejuni O:41 strains associated with Guillain-Barré syndrome exhibit mimicry of GM1 ganglioside. *Infect Immun* 1998; 66: 3649-55.
37. MORI M, KUWABARA S, MIYAKE M *et al*.: Haemophilus influenzae has a GM1 ganglioside-like structure and elicits Guillain-Barré syndrome. *Neurology* 1999; 52: 1282-84.