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Jean-Michel Vallat, Nathalie Deschamps, Laurence Richard, Laurent Magy, Jérôme J. Devaux, Stéphane Mathis

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**Widenings of myelin lamellae in an IgM polyneuropathy without
anti-MAG activity, responsive to treatment**

Abstract

We report the case of a very severe polyneuropathy that has developed over a few months, in association to an IgM monoclonal gammopathy without anti-MAG antibodies ; WML were frequently observed by electron microscopic examination of a nerve biopsy. Usually, in anti-MAG neuropathy, the response to immunotherapies is not optimal, but in this case the combination of plasma exchanges and rituximab has been quite effective.

Key words: polyneuropathy; nodoparanodopathy; internodopathy; anti-MAG; rituximab.

Introduction

The so-called 'widenings of the myelin lamellae' (WML) are ultrastructural lesions of nerves at the levels of internodal and paranodal areas which are generally observed in nerve biopsy (NB) from patients with a demyelinating polyneuropathy associated to an IgM monoclonal gammopathy with antibodies against 'myelin associated glycoprotein' (MAG). This gammopathy is often of undetermined significance (MGUS) or may correspond to Waldenström disease.¹ The very high incidence (about 70%) of anti-MAG antibody activity in patients with IgM monoclonal gammopathy and a demyelinating neuropathy is well known.²

Methods

We report here the case of a very severe polyneuropathy associated to an IgM monoclonal gammopathy without anti-MAG antibodies. A NB was performed after written consent of the patient.

The NB specimen (sural nerve) was divided into three portions. Paraffin sections of the first portion were examined using standard methods. Transverse cryostat sections of the second portion were examined by direct immunofluorescence microscopy using rabbit monospecific to human IgA, IgG, IgM, kappa and lambda light chains and to C19, C3c, C3d, and C5 components of complement (*Dakopatts, Globstrug*, Denmark). The third fragment was immediately fixed in 5% glutaraldehyde and postfixed in 1 % osmium tetroxide. Epon-embedded semithin and ultrathin sections were prepared for light and electron microscopic examination.

Results

Clinical data

A 72-year-old woman with a history of arterial hypertension and diabetes mellitus (type 2) presented a few paresthesia of both hands for 6 months associated with grip difficulties (**Figure 1A**). Three weeks after the paresthesia, she developed some moderate neuropathic pain of the lower limbs (predominantly distal). After 6 months, the clinical examination found predominant motor deficit distally of the upper limbs (total MRC score: 90/100), hypoesthesia (in all modalities) of the 4 extremities and absent ankle-deep tendon reflex: the total 'Overall Neuropathy Limitations Scale' (ONLS) score was 3/12. No tremor was observed. The electrodiagnostic study found a (predominantly) demyelinating sensorimotor polyneuropathy with axonal loss (**Figure 1B**). The biological assessment showed an IgM-kappa monoclonal gammopathy (3.18 g/L; normal value: 0.4-2.4 g/L) and the search for anti-MAG and anti-ganglioside antibodies was negative (it was done several times on several serum samples in two different specialized immunology laboratories). A first course of intravenous immunoglobulins (IVIg), at a total dosage of 2 g/kg (5 days), was introduced without noticeable improvement.

Three weeks later, she presented rapid worsening of the symptoms, especially an increase of the motor weakness in all four limbs (associated with binocular diplopia and swallowing disorders) and generalized absent deep tendon reflex: total 'Medical Research Council' (MRC) score for motor testing was 66/100; total 'Overall Neuropathy Limitations Scale' (ONLS) was graded 6/12. There was also worsening of the electrophysiological parameters: severe axonal loss and more demyelinating features (**Figure 1B**). The biological assessment was updated, showing the persistence of the monoclonal gammopathy; HbA1c was 7%; antibodies against MAG, sulfo-glucuronyl-paragloboside (SGPG) and gangliosides were still negative, with normal immunological assessment; the viral serologies were negative; no mutation of the *PMP22* gene was found. The lumbar puncture showed 1

leucocyte/mm³, high protein level (124 g/dL), absence of intrathecal synthesis and negative viral PCR in the cerebrospinal fluid. The whole-body CT-scan and ¹⁸fluorodeoxyglucose positron emission tomography were unremarkable. The bone marrow aspiration was performed twice, revealing no lymphocytic infiltrate, but the *MYD88* L265P mutation was detected in the bone marrow. In this context, a neuromuscular biopsy was performed, showing argues for a peripheral nerve demyelination (see details below), associated with muscle atrophy (neurogenic pattern).

Three new monthly courses of IVIg were carried out at a dosage of 2 g/kg/5 days, associated with intravenous corticosteroids (400 mg/day for 3 days, then oral administration at a dose of 1 mg/kg/day for three weeks and gradual decrease to 20 mg/day). Two weeks and half later, because of clinical worsening (tetraplegia; total ONLS: 11/12) and the occurrence of dyspnea, IVIg were switched for plasma exchange (PLEX): 6 sessions were carried out during 10 days; then, anti-CD20 treatment (rituximab) was immediately performed: one intravenous course (1,000 mg), then a second course (1,000 mg) 2 weeks later (**Figure 1A**). We observed onset of improvement 2 weeks after the second infusion of rituximab; the patient was able to walk 2 weeks later (total ONLS: 5/10). Six months later, she was only treated by oral corticosteroids (20 mg/day) and gabapentine: there was no more motor weakness, only mild proprioceptive ataxia and paresthesia in the sole of the feet (total ONLS: 1/12; **Figure 1A**). A third course of rituximab was performed 6 months after the second one and at that time the patient had stopped steroids and complained only of a few distal paresthesia of the inferior limbs.

Pathological data

Light microscopy: No cellular infiltrates were observed in the endoneurium and the density of myelinated fibers was significantly diminished on semithin and ultrathin sections. Deposits of IgM, Kappa light chains and complement (C3d) were observed in many myelin sheaths on direct immunofluorescence (figure 2). Indirect immunofluorescence (which tests reactivity of the patient's serum against normal human normal nerve) showed a positive myelin staining with anti-IgM and anti-C3D at the time of NB, when the patient presented tetraplegia; this positivity was still present 6 months later when the clinical state of the patient was close to normal.

Electron microscopy: A few demyelinating and totally naked axons were observed; some myelinated fibers were invaded by a macrophage which had penetrated the cytoplasm of the Schwann cell and was overloaded with myelin debris. Around 38% of myelinated fibers exhibited regular widenings of their outermost lamella (WML). At high magnification, these WML, which seemed empty, consisted of two thin lamellae stuck to dense myelin sheaths which correspond to the separated leaflets of the intermediate line. The spacing between two normal dense lines of the myelin sheaths was around 13 nm, and around 27 nm between the abnormal widenings. Immuno-electron microscopy confirmed the presence of IgM-Kappa light chains (figure 2) and complement (C3d) in the WML and also revealed IgM and Kappa light chain deposits in the endoneurial space between Schwann cells.

Discussion

In peripheral neuropathies induced by IgM monoclonal gammopathy (in both MGUS or Waldenström disease) with anti-MAG activity, the ultrastructural examination of NB usually detects (in a significant number of remaining myelinated fibers) WML which are characterized by abnormal enlarged spaces (about 23 nm wide between the separated leaflets

of the intermediate line); these spaces contain an electroluminescent material. In most fibers, these widenings are few, restricted to the outermost lamellae¹ and usually ultrastructurally observed only by a careful examination at high magnification. In the case we discuss here, the lesions were quite frequent (almost 40% of the remaining myelinated fibers) and often concerned a significant number of peripheral myelin lamellae.

MAG is said to be localized to the regions of the myelin sheath in which membranes are uncompacted such as the periaxonal area, the Schmidt-Lanterman areas and the paranodes where each terminal myelin loop is separated from its neighbors by a space of 12-14 nm, a distance associated with the presence of MAG in the membranes.^{3,4} As in our case, WML concern also the terminal myelin loops of the paranodal regions, particularly before the appearance of extensive demyelination to internode areas, probably by activation of the complement pathway.^{5,6}

These regular widenings are thought to be almost specific of polyneuropathies associated with anti-MAG antibodies ; however, they have been encountered in other (albeit rare) conditions, such as in 'experimental acute neuropathy' (EAN) and Marek's disease,⁷ or in cultures of peripheral myelin treated with the serum of patients with Guillain-Barré syndrome (GBS) or serum from animals with EAN.^{8,9} In human peripheral neuropathies, such abnormalities have been observed in a case of myeloma,¹⁰ in a benign IgG monoclonal gammopathy and in a case of subacute inflammatory polyradiculoneuropathy (in a diabetic patient).^{1,11} In our cohort of 60 patients with anti-MAG neuropathy,¹² WML constantly coexisted with macrophage-associated demyelination, a feature that is considered typical of 'Chronic Inflammatory Demyelinating Polyneuropathy' (CIDP), as described by others.¹³ It has to be stressed that the patient with IgM monoclonal gammopathy we discuss here, presented the same ultrastructural characteristics: in this patient, WML concerned about 40% of myelinated fibers left, with active demyelination induced by macrophages; however, anti-

MAG antibodies (and antibodies against its cross-reactive SGPG) were never found after several dosages (by two different specialized laboratories). It is probable that the monoclonal gammopathy of this patient induces the same nerve lesions, but due to different antibodies (against a different myelin antigen than MAG or SGPG).

For our patient, the subacute onset of the symptoms was gradual (within a few months), mainly motor, affecting the upper and lower limbs equally with a subsequent acute worsening that was quite rapid (within a few weeks) and particularly severe. It is known that polyneuropathy induced by MAG is usually a chronic, slowly progressive large-fiber sensory-motor polyneuropathy, usually affecting initially the lower extremities and worsens slowly over one or two decades, usually with poor response to IVIg treatment (contrary to what is classically observed in CIDP).² Actually, the evidence for the efficacy of IVIg in anti-MAG polyneuropathy is limited (usually class II evidence),¹⁴ but our personal experience is that some patients may have a good response to IVIg,^{12, 15} warranting its use as a first-line option.¹⁶ In case of inefficacy of IgIV, PLEX may be useful, this treatment being temporarily effective in approximately half of the patients with anti-MAG neuropathy, both alone and in combination with other therapies (class IV evidence).¹⁷ Finally, two controlled studies (using different scales and endpoints) demonstrated a positive benefit of rituximab in anti-MAG neuropathy,^{18, 19} so this is nowadays a common treatment in such polyneuropathy (usually as a second line treatment, after IVIg and PLEX), although its efficacy is limited (about 30 to 50% of cases).^{2, 20} In anti-MAG neuropathy, this chimeric murine-human monoclonal antibody against CD20 (causing depletion of circulating B cells) does not affect the antibodies producing plasma cells, so neurological improvement is typically delayed beginning about three months after treatment and is clear six months after therapy,¹⁸ coinciding with the slow reduction of anti-MAG antibodies.

It is interesting to see that our patient (IgM dysglobulinemic neuropathy without anti-MAG activity) presented an electrophysiological and pathological pattern consistent with an anti-MAG polyneuropathy, but with a subacute/acute evolution (atypical for this kind of polyneuropathy). Because of the rapid and severe worsening, different treatments were successively prescribed during a period of a few weeks. So, it is not easy to precisely identify which therapeutic modality is responsible for the fast and impressive improvement: it could be due to PLEX, rituximab probably playing a role in the maintenance of the subsequent stable clinical condition. How long should be maintained the rituximab treatment (which has been quite effective) has to be discussed. It has to be stressed that, after a few months of a very significant clinical improvement, the serum of the patient is still very active on myelin of frozen normal human peripheral nerve by indirect immunofluorescence: it seems to us that, as long as this activity persists, we should maintain this immunomodulatory treatment.

In conclusion, this polyneuropathy associated to an IgM gammopathy without anti-MAG antibody is atypical for several reasons. Concerning the evolution, severe motor symptoms and signs developed over several months, with no response to IVIg and steroids; nevertheless, the improvement was dramatic after PLEX (two weeks after the second administration of rituximab). Electrophysiological data were compatible with an anti-MAG neuropathy and the EM study revealed typical WML with the presence of IgM and Kappa chain deposits. So, the non-anti-MAG IgM of this patient which induces the same nerve lesions than those observed in anti-MAG neuropathy could be linked to other antibodies (against a different myelin antigen than MAG or SGPG). Nerve biopsy was decisive to confirm the dysimmune process and finally was very useful to discuss and establish an efficient treatment.

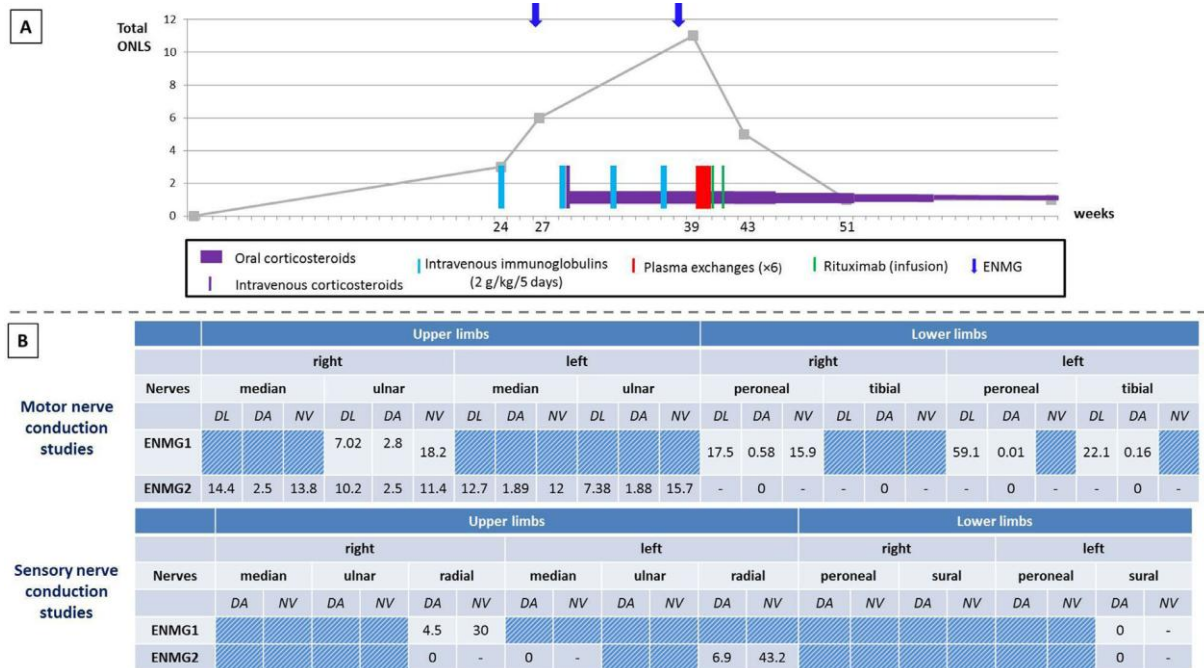


Figure 1. Clinical and electrophysiological follow-up of the patient.

A: schematic evolution of the ‘Overall Neuropathy Limitations Scale’ (ONLS) score and representation of the various treatments used.

B: Results of the motor and sensory nerve conduction studies during 2 successive electrodiagnostic studies (DA: distal amplitude; DL: distal latency; ENMG: electroneuromyogram; MNV: motor nerve velocity; m/s: meter per second; ms: millisecond; µV: microvolt; mV: millivolt; SNV: sensory nerve velocity).

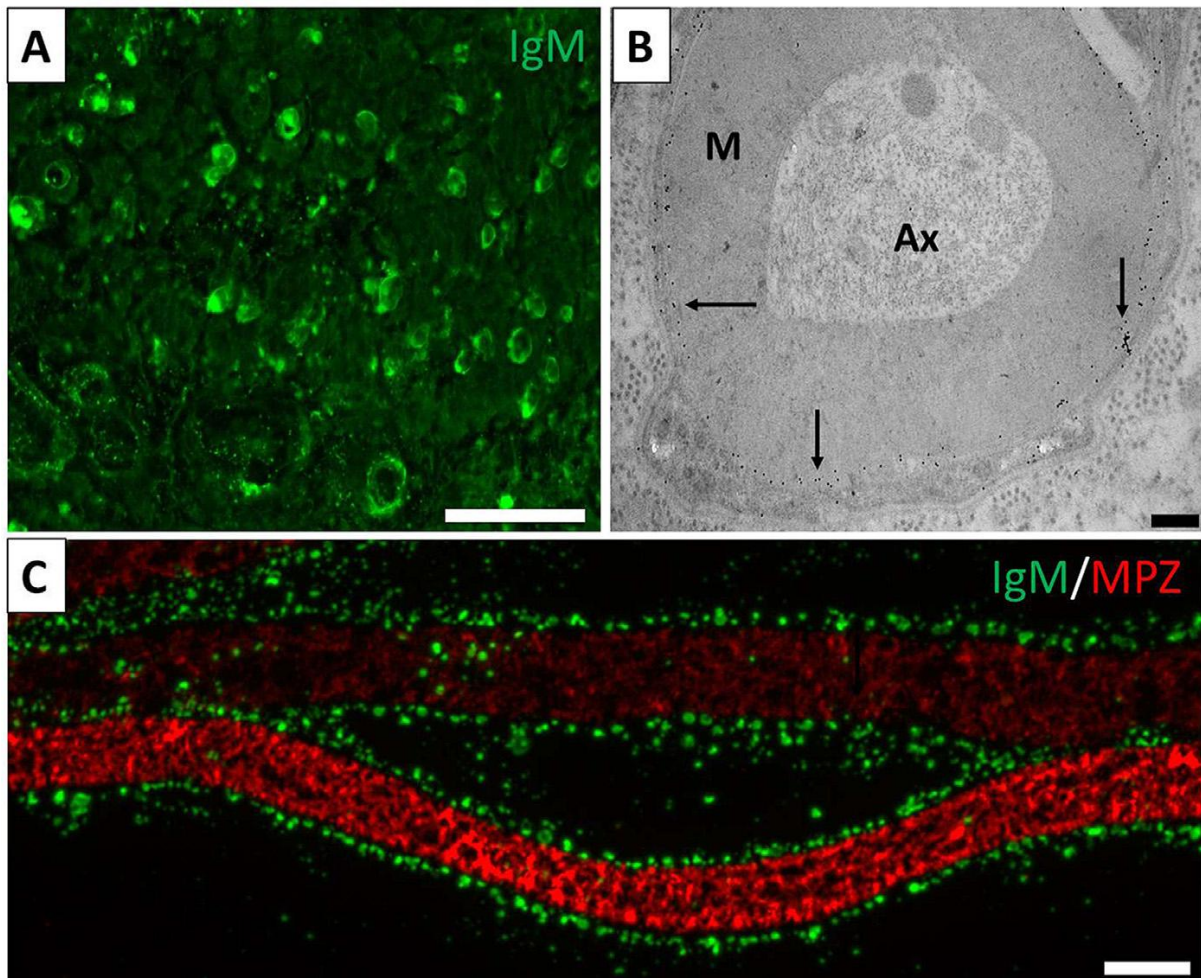


Figure 2. IgM deposits around myelin sheath . A : Frozen section of the patient’s sural nerve biopsy: most of the myelin sheaths left are specifically immuno-labeled by fluorescent IgM antibody (scale bar: 50 μ m). B : Post-embedding immunolabeling of IgM on an ultrathin cross section of the patient’s sural nerve biopsy revealing the presence of IgM deposits on the outer myelin sheath layers (black arrows) (axe: axon; M: myelin; scale bar: 500 nm). C : Mouse sciatic nerve fibers were stained with the patient’s serum and revealed for human IgM (green) and with a rabbit antibody against myelin protein zero (MPZ; red) (scale bar: 10 μ m).

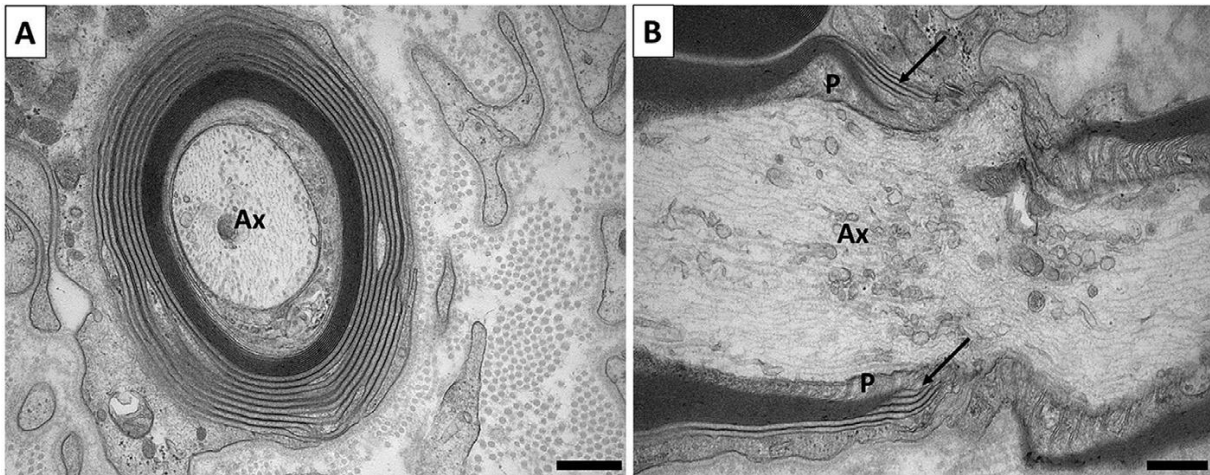


Figure 3. Evidence of widenings of myelin lamellae . A : Cross-sectional electron microscopy micrograph of a myelinated axon showing extensive regular enlargement of the inter-period line between the outer myelin lamellae (axe: axon; scale bar: 500 nm). B : Longitudinal section of a myelinated axon showing widenings of myelin lamellas at the paranodal region (black arrows) (axe: axon; P: paranode; scale bar: 500 nm).

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