Waldenström's Macroglobulinemia and Peripheral Neuropathy: A Clinical and Immunologic Study of 25 Patients

By Koussay Dellagi, Pierre Dupouey, Jean Claude Brouet, Agnès Billecocq, Danielle Gomez, Jean Pierre Clauvel, and Maxime Seligmann

We investigated, by indirect immunofluorescence, the binding of monoclonal IgM to human peripheral nerve in 25 patients with Waldenström's macroglobulinemia and peripheral neuropathy. In 10 cases (40%), an antibody activity against the myelin sheaths was demonstrated. The reactivity was mediated by the (Fab')2 fragments of the IgM. Prior delipidation of nervous tissue was needed to allow full expression of the target antigen(s). In nine cases, the IgM reacted with both peripheral and central myelin of primates, but not mouse or rabbit nervous tissue. In one case, the IgM reacted only with human peripheral nerve. The patients, whose IgM had an antibody activity to myelin antigen(s), had some distinct hematologic and neurologic features. The peripheral neuropathy always antedated the hematologic symptoms by several years. The serum level of the monoclonal IgM was low in all cases, and overt lymphoid malignancy was frequently absent. In other patients with neuropathy, the monoclonal IgM, which lacked antimyelin antibody activity, displayed either cross-idiotype antigenic determinants or anti-intermediate filament antibody activity. These results taken together emphasize the heterogeneity of antibody activity of the monoclonal IgM from patients with Waldenström's macroglobulinemia and peripheral neuropathy.

A PERIPHERAL NEUROPATHY occurs in about 5% of patients with Waldenström's macroglobulinemia (WM). The pathogenesis of this complication is largely unknown, although the role of a serum hyperviscosity, a bleeding tendency, a lymphoid infiltration of nerves, and amyloidosis have been discussed. A direct role for monoclonal IgM is supported by the demonstration of IgM deposits on nerve sections from patients with neuropathy, as well as by the binding of the IgM to nerve structures. That the monoclonal IgM is involved in the pathogenesis of the neuropathy through its antibody activity has been recently supported by the finding that the IgM from some of the patients with neuropathy shared crossidiotypic antigens and, therefore, presumably had a similar antibody activity. Moreover, Latov et al. showed that monoclonal IgM from four patients possessed an antibody activity directed to myelin antigens.

We report the clinical and immunologic data from a group of 25 patients with macroglobulinemia and peripheral neuropathy. In 40% of the cases, the patient's monoclonal IgM was able to bind to human or primate myelin sheaths, as shown by immunofluorescence on fixed sections of peripheral nerve or brain; these patients whose IgM had an antibody activity to myelin antigens had some distinct hematologic and neurologic features.

MATERIALS AND METHODS

Patients

Twenty-two patients were followed in the department of hematology and immunopathology of Hôpital Saint Louis, Paris. We also obtained a detailed clinical chart from 3 patients followed in other institutions. An electrophysiologic study was performed in 10 cases, a nerve biopsy in 12, and a cerebral spinal fluid (CSF) analysis in 14. All patients underwent a bone marrow aspiration, and 20 had one or several bone marrow biopsies. Pertinent clinical and laboratory data are listed in Table 1.

Purification of Monoclonal IgM and Fragments

Sera from patients suffering from WM with (25 cases) or without (50 cases) peripheral neuropathy were stored at -20°C prior to use. Purification of the monoclonal IgM was achieved by ammonium sulfate precipitation followed by Sepharose 6B chromatography. Fab and Fc or F(ab')2 fragments of the purified IgM were obtained, respectively, after trypsin or pepsin digestion. In two cases, F(ab')2 fragments were crosslinked with glutaraldehyde.

Immunofluorescence Studies

Human sciatic nerve and brain (corpus callosum) were obtained less than 8 hr after death. Sciatic nerves from other species [monkey (chimpanzee), rabbit, and mouse] were also studied with the monoclonal IgM reactive with human nerve. Control studies were performed on liver or kidney tissues. The samples were either immediately frozen in isopentane or fixed in 4% formalin-phosphate-buffered saline (14 hr at 4°C), washed extensively during 48 hr in 8% saccharose-phosphate-buffered saline, and embedded in paraffin. Frozen or paraffin sections were cut at 4-5 μ. For indirect immunofluorescence studies, tissue sections were pretreated with 4% bovine albumin for 20 min and incubated with various dilutions of sera or purified IgM and fragments for 30 min, washed, and finally stained with fluorescein-conjugated rabbit IgG to human Ig (Institut Pasteur Production) or to Ig heavy (Wellcom Laboratories) or light chains (Behringwerk Laboratories, Marburg-Lahn, Germany). Fluorescence was graded from 0 to + + + +. The titer of the sera was expressed as the inverse of the last dilution giving a + + fluorescence.
Table 1. Distinctive Clinical and Biologic Features of 25 Patients With Neuropathy

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Mean age (range)</td>
<td>61 yr (47–72)</td>
<td>64 yr (51–80)</td>
</tr>
<tr>
<td>Sex M/F</td>
<td>9/1</td>
<td>12/3</td>
</tr>
<tr>
<td>Mixed sensory</td>
<td></td>
<td></td>
</tr>
<tr>
<td>motor neuropathy</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>Pure sensory</td>
<td></td>
<td></td>
</tr>
<tr>
<td>neuropathy</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Pure motor neuropathy</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Neurorphy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>as presenting symptom</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Mean duration of</td>
<td></td>
<td></td>
</tr>
<tr>
<td>neurologic symptoms (range)</td>
<td>7 yr (1–15)</td>
<td>3.5 yr (1–10)</td>
</tr>
<tr>
<td>Monoclonal IgM levels:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10 mg/ml</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>&gt;10 mg/ml</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Overt bone marrow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>plasmacytic infiltration</td>
<td>4</td>
<td>14</td>
</tr>
</tbody>
</table>

Group I: IgM with antibody activity to myelin; group II: IgM without antimyelin antibody activity.

For direct immunofluorescence, 3 monoclonal IgM were conjugated to fluorescein and used at a concentration of 50 μg/ml. To compare the antibody specificity of various IgM, blocking experiments were performed: the conjugated IgM were used to stain nerve sections previously incubated overnight at 4°C with unconjugated monoclonal IgM (250 μg/ml) or sera (diluted 1/30) from patients with neuropathy.

In some experiments, the monoclonal purified IgM at a concentration of 20 μg/ml was absorbed with peripheral nerve myelin at 4°C for 4 hr. Myelin was obtained by sucrose flotation.

RESULTS

Study of the Binding of Monoclonal IgM to Myelin Sheaths

By indirect immunofluorescence, sera from 10 of the 25 patients with peripheral neuropathy reacted strongly with sections of human nerves fixed in formalin and embedded in paraffin. The light chains of the serum monoclonal IgM were kappa in all these cases, and this reactivity was detectable only with the anti-mu and anti-kappa monospecific sera. The same pattern of reactivity was observed with the purified IgM either by indirect or by direct immunofluorescence. The titer of antimyelin activity ranged from 2,000 to 30,000. No reactivity was detectable with the purified Fc, Fab, or F(ab')2 fragments of the two IgM studied. The negative results with the latter fragments are most likely explained by their low affinity, since glutaraldehyde-crosslinked F(ab')2 fragments of these IgM (but not from control IgM) did bind to the nerve sections. The staining of myelin sheaths was almost abolished by previous absorptions of the monoclonal IgM with purified myelin. These IgM had no reactivity on human liver and kidney sections.

All positive sera but one stained cross-sections of the whole myelin sheath (Fig. 1). However, the intensity of the staining varied from one sheath to another, the large myelinated fibers being the most intensively stained. On longitudinal sections, the staining was either linear, on both sides of the axons, or restricted to the Schmidt-Lantermann incisure. These two patterns coexisted on the same section. These nine sera stained, although more weakly, the myelin sheaths of the human corpus callosum. The last serum gave a different pattern of fluorescence on peripheral nerves, since it stained only the inner and outer border of the myelin sheaths, giving two concentric rims of fluorescence. In some sections, the outer border clearly involved Schwann cell bodies. This serum did not react with central nervous system (CNS) myelin.

The nine sera that were positive on human central and peripheral myelin also stained the peripheral nerves from monkeys in an identical fashion, but were unreactive with mouse or rabbit nerves. The one serum that stained only the peripheral myelin was negative on monkey, rabbit, and mouse nerves.

To compare the specificity of these ten positive monoclonal IgM, blocking experiments were performed as follows. The ability of unconjugated IgM to abolish the staining of myelin sheaths by a given fluoresceinated IgM (IgM Pec.) was studied. The staining by the fluoresceinated IgM was strongly inhibited by the homologous unconjugated IgM as well as by five other IgM or sera. Four sera (including the one that stained Schwann cells) had no effect. No inhibition was observed with three IgM from patients with neuropathy, which did not react with myelin by fluorescence.

No staining on nerve sections was observed with 10 normal sera. In a control group of 50 sera from patients with WM without neuropathy, 3 sera stained the myelin sheaths. Two of these IgM also stained kidney and liver sections, and therefore their reactivity with tissues was clearly different from that observed with IgM from patients with neuropathy.

It should be stressed that formalin fixation was needed to allow the full expression of the antigen(s) reactive with the monoclonal IgM. Indeed, no reactivity was detectable with myelin antigens on sections of fresh-frozen human nerves. In this latter condition, two additional monoclonal IgM from this series of patients stained Schwann cells (and not myelin sheaths); as reported previously, these IgM reacted with vimentin intermediate filaments of the cytoskeleton.

Clinical Studies

In view of the above immunologic study, patients with WM and neuropathy could be distinguished
according to the presence of a monoclonal IgM with (group I) or without (group II) antibody activity to myelin antigens. Several clinical features were not distinctive between the two groups: the mean age and sex ratio were similar; the peripheral neuropathy was usually of the mixed sensory motor type. An increased proteinorachia was observed in all cases but one. The conduction velocity was always reduced and distal latencies prolonged. On nerve biopsy, the salient feature was a loss of myelinated fibers of variable intensity. In addition, two patients from group II had perivascular lymphoplasmacytic infiltrates. No amyloid deposits were found; however, two patients from group II had amyloidosis on kidney or muscle biopsies, respectively.

Contrasting with these common features, some differences were found between the two groups. Symptoms related to the peripheral neuropathy were the presenting complaint of all group I patients; they lasted for up to 8 yr (mean 4 yr) before the finding of the serum monoclonal IgM. This is in contrast with group II patients, where neurologic symptoms appeared 1–5 yr (mean 2.5 yr) after the diagnosis of WM in 7 of 15 patients. In group I, a single patient, followed presently for 8 yr, had a pure sensory neuropathy of the four limbs confirmed by electrophysiologic studies. This patient's IgM reacted on nerve with a unique pattern, since it stained only the inner and outer border of the myelin sheath and the Schwann cell. Nine patients had a sensory motor polyneuropathy. Paresthesias was the major complaint; there was a symmetric and distal sensory loss affecting the four limbs. Objective decrease of pain sensation was rare, but there was a marked impairment of proprioceptive sensitivity in most patients, with ataxia in four. In eight patients, the decrease of muscle strength was moderate and usually occurred 2–5 yr after the sensory symptoms. In one patient, motor symptoms were prominent and appeared abruptly. A diffuse areflexia was noted in all nine patients. The mean follow-up for these patients is 7 yr (one to 15 yr).

Among group II patients, 2 presented with a pure motor neuropathy (assessed in one patient by electrophysiologic studies) lasting presently for 4 to 12 yr, respectively. Thirteen patients had a sensory motor polyneuropathy; general areflexia was rare in this group (3 patients) and neurologic symptoms were rather asymmetric in 4 cases. The mean follow-up

Fig. 1. Indirect immunofluorescence on formalin-fixed human sciatic nerve with the monoclonal IgM from a patient with Waldenström's macroglobulinemia and peripheral neuropathy. (A) Cross-section: the whole myelin sheath is stained. There is no staining of the axons or the endoneural interstitial tissue. (B) Longitudinal section. (Scale: 1 bar = 20 µ.)
since the onset of the neurologic symptoms is 3.5 yr for this group (1–10 yr).

All group I patients had a low level of monoclonal IgM (less than 10 mg/ml) on electrophoresis. This was in sharp contrast with group II patients, whose serum IgM levels were higher than 10 mg/ml in 10 of 15 cases. Bone marrow lymphoplasmacytic infiltration was detected in all patients from group II but one, whereas it was detectable in only 40% of the patients from group I. In these latter cases, the lymphoid infiltration of the bone marrow was moderate, and several bone marrow biopsies were often needed to definitely establish the existence of a lymphoid proliferation.

DISCUSSION

A peripheral neuropathy occurs in about 5% of WM patients.13 This incidence is much higher than in chronic lymphocytic leukemia. Therefore, a direct role for the monoclonal IgM in the pathogenesis of the neuropathy has been suggested on various grounds. We have presented the results of a clinical and immunologic study performed in 25 WM patients with neuropathy. To explore the possibility that the monoclonal IgM possessed an antibody activity against some nerve antigens, we studied the binding of the monoclonal IgM by direct or indirect immunofluorescence on human nerve sections. Ten monoclonal IgM reacted with myelin sheaths on formalin-fixed paraffin sections but not on frozen sections, indicating that delipidation is needed for the full expression of the antigenic determinant(s), which is therefore nonlipidic. No reactivity was noted on liver or kidney sections. In 9 cases, the monoclonal IgM stained the whole myelin sheath. Most likely, the binding of the IgM occurred via the antigen binding site of the molecule, since Fc fragments were unreactive whereas F(\(\alpha\)'B), fragments (only after being crosslinked by glutaraldehyde treatment) did bind to nerve. This last finding is probably explained by a low affinity of the monoclonal IgM for its antigen, a finding not uncommon for monoclonal Ig with autoantibody activities.14 In these 9 cases, the IgM reacted with myelin from both the central and peripheral nervous system. Such a reactivity has been recently reported.4 Interestingly, we found that these monoclonal IgM reacted with human or primate but not with rabbit or mouse myelin. Recently, Latov et al. identified a 100,000-dalton protein of peripheral and central myelin as the target antigen of 4 different monoclonal IgM.7 Whether IgM for most patients with neuropathy react with this antigen is presently unsettled. Our results suggest that different antigenic determinants may be involved, since the binding of a given IgM was not inhibited by three of the eight other positive IgM in blocking immunofluorescence experiments. Moreover, the serum from one patient had a unique pattern of fluorescence on nerve sections, since it stained the inner and outer border of the myelin sheath from peripheral nerves only, indicating a possible reactivity with an antigen carried by the Schwann cell.

It is of note that one monoclonal IgM of 50 from the control group of patients without clinical or electrical peripheral neuropathy exhibited a similar reactivity with myelin sheaths. Although the incidence of this antibody activity is significantly different in the two groups, this finding questions the pathogenic role of the monoclonal IgM. This case is reminiscent of occasional patients with WM and an autoantibody activity of the monoclonal IgM directed either to \(\alpha\)II antigens or to IgG (mixed cryoglobulinemia) who remain free of clinical symptoms for years. On the other hand, it is conceivable that the IgM is not sufficient by itself to provoke a nerve injury. Since pentameric IgM does not cross the epineurium barrier under normal circumstances, other factors may play a role, for instance a local synthesis of IgM molecules by lymphoplasma cytic infiltrates,15 the circulation of the monomeric form of the IgM,16 or a simultaneous nerve insult caused by virus or drugs. Although direct immunofluorescent studies on nerve biopsies could be obtained in 3 patients only, it is of note that the single nerve that contained IgM deposits belonged to a group I patient, whereas no IgM was observed in the two group II patients studied.

Those patients whose monoclonal IgM had an antibody activity directed to myelin shared some distinct hematologic and neurologic features. In all these patients, the peripheral neuropathy antedated the hematologic symptoms by several years, whereas in nearly half of the group II patients, the neuropathy developed several years after the diagnosis of macro globulinemia. All but one patient from group I had a symmetric sensory motor polyneuropathy involving the four limbs, often with prominent sensory symptoms. One patient had a pure sensory neuropathy; the IgM from this patient has a unique reactivity on nerve sections that suggested that it might react with some antigen of the Schwann cell. Finally, group I patients had significantly lower IgM levels than group II patients, and in half of the cases, had no detectable lymphoid proliferation. This situation is close to that of chronic cold agglutinin disease, where overt lymphoid malignancy may develop several years after the diagnosis. In such cases, the autoantibody activity of the monoclonal IgM may explain why the disease is diagnosed at a time when the tumor burden is very small.

It must be stressed that the lack of detectable
reactivity of the monoclonal IgM with myelin (group II patients) does not rule out the possibility that the nerve injury is immunologically mediated. Indeed, two patients from this group had a monoclonal IgM with an antibody activity against vimentin intermediate filaments; although not organ specific, this antigen is characteristic of Schwann cell intermediate filaments. The pathogenesis of the neuropathy in these two patients is debatable, but it is worth noting that a peripheral neuropathy was transmitted to mice by injection of human monoclonal Ig with an antibody activity to some cytoskeleton proteins. Lastly, we have previously shown that the monoclonal IgM from six patients with neuropathy shared crossidiotypic antigens; by analogy with other crossidiotypic systems shared by monoclonal IgM with a defined antibody activity, this finding suggests that these IgM had a similar antibody activity that is yet undefined, since none of these six crossidiotypic IgM reacted with myelin antigens by immunofluorescence. Conversely, those IgM that react with myelin do not belong to the crossidiotypic group. It is of interest that four of the six patients belonging to the crossidiotypic group also had a symmetric sensory motor neuropathy that antedated the diagnosis of WM and had low serum IgM levels, all features shared with group I patients. Taken together, these results show that the monoclonal IgM of 18 of 25 patients with WM and neuropathy had some unique antigenic characteristics or antibody activity. Moreover, although the overall clinical course of the neuropathy is somehow similar, the immunologic data indicate some heterogeneity in the antibody activity of the monoclonal IgM. The pathogenesis of the neuropathy may therefore differ from patient to patient. Although these results strongly suggest that the neuropathy is mediated by the monoclonal IgM, experimental data are clearly needed to definitely assess the mechanism of the neuropathy.

The patients from the present series were studied during various periods and received no uniform treatment. It is therefore difficult to correlate the immunologic findings and the efficiency of therapy. Improvement of the neuropathy always coincided with a long-lasting decrease of the IgM level, which in three patients was obtained under therapy with alkylating agents and intermittent plasmaphereses. This treatment failed in three other patients. More recently, extensive plasmaphereses with a cell separator were performed in five patients (three of whom had a monoclonal IgM displaying an antibody activity directed to myelin antigens) with objective improvement of the neuropathy in four cases (the one failure was observed in a group I patient). Strikingly, two patients with a neuropathy lasting for more than 10 yr definitely improved. However, from the present data, it is obvious that the therapeutic strategy in such patients cannot rely only on the results of the immunologic studies.

ACKNOWLEDGMENT

We thank Dr. A. Delannoy and Dr. Bierling for referring the sera of some of the patients and Dr. F. Danon for performing immunoelectrophoretic analysis of the sera.

REFERENCES

Waldenstrom's macroglobulinemia and peripheral neuropathy: a clinical and immunologic study of 25 patients

K Dellagi, P Dupouey, JC Brouet, A Billecocq, D Gomez, JP Clauvel and M Seligmann