Cross-idiotypic Antigens among Monoclonal Immunoglobulin M from Patients with Waldenström's Macroglobulinemia and Polyneuropathy

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ABSTRACT The monoclonal immunoglobulin (Ig)M from 5 to 16 patients with Waldenström's macroglobulinemia and a polyneuropathy shared cross-idiotypic antigenic determinants as demonstrated by hemagglutination and hemagglutination inhibition experiments as well as by precipitin reactions. This reactivity was located to the Fab (and not Fc) fragment of the protein. The IgM from 73 patients with macroglobulinemia but without neuropathy all gave negative reactions. In contrast, the monoclonal IgG from a patient with polyneuropathy also possessed similar idiotypic determinants. Since cross-idiotypic determinants are usually related to the combining site of a monoclonal Ig, this finding suggests that the monoclonal Ig of these patients may mediate the nerve injury via their antibody activity, which could be directed either to a nerve antigen or to some component involved in the pathogenesis of the neuropathy.

INTRODUCTION

In man idiotypic determinants common to monoclonal immunoglobulin (Ig) from different individuals have been observed in two different circumstances. First, cross-idiotypic determinants have been demonstrated among monoclonal Ig that had an identical antibody activity (1, 2), and second, common idiotypes have been found, although rarely, on monoclonal Ig purified from the serum of different individuals within a family (3). The former idiotypic determinants have been shown conclusively to be related to the structure of the combining site of the monoclonal Ig, whereas the latter may be tentatively assigned to a common genetic background.

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A polyneuropathy occurs in about 5% of patients with Waldenström's macroglobulinemia (WM)[1] (4). The pathogenesis of this severe complication is largely unknown. However, IgM deposits in nerves were demonstrated in some cases, (5–10) raising the intriguing possibility that the IgM mediates the nerve injury, possibly through an immunological interaction. We have therefore investigated the possibility that the monoclonal IgM from these patients may have cross-idiotypic antigenic determinants and report in this paper that indeed a subgroup of patients with WM and neuropathy (interestingly, including two sisters) does in fact share cross-reactive idiotypes.

METHODS

Sera from 16 patients with WM and a sensori-motor polyneuropathy and from 70 patients with WM without neurological symptoms were studied as well as sera from patients with monoclonal IgG (three cases) or IgA (one case) who also suffered from a peripheral neuropathy. Purification of the monoclonal IgM was achieved in 19 cases by euglobulin or ammonium sulfate precipitation followed by Sepharose 6B (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) chromatography. The ascending part of the first protein peak eluted from the column was devoid of α2-macroglobulin by immunodiffusion at a concentration of 5–10 mg/ml and used in all further experiments. Polyclonal IgM were similarly purified from the serum of a patient with African trypanosomiasis. Polyclonal IgG were obtained from Cohn fraction II by DEAE cellulose chromatography. Monoclonal IgG from patient Ha were separated from the 19S fraction by preparative ultracentrifugation in a sucrose density gradient. Fab and Fc fragments from IgM Gen and Ngu were obtained by tryptic digestion according to Plaut and Tomasi (11) and separated by Sepharose 6B chromatography.

Antisera to IgM Vin were obtained in rabbits. The protein Vin was isolated by ammonium sulfate precipitation, Sephadex

[1] *Abbreviation used in this paper*: WM, Waldenström's macroglobulinemia.

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G-200 (Pharmacia Fine Chemicals, Inc.) chromatography and Pevikon (Mercer Consolidated Corp., Yonkers, N. Y.) block electrophoresis. Rabbits were immunized with the purified IgM in Freund's complete adjuvant in foot pads and then boosted at regular interval subcutaneously. 1 ml of the resulting antiserum was absorbed with 0.1 ml normal serum, 2.5 mg IgM (four different monoclonal proteins from patients free of neurological symptoms), and 2.5 mg polyclonal IgG. Direct hemagglutination tests were performed with sheep erythrocytes coated with the purified IgM by the chromium chlorid method (12) and read on opalin plaques.

Inhibition of hemagglutination was assayed by incubating one drop of the properly diluted anti-idiotypic serum with purified IgM or serum for 15 min at 22°C before testing. All reagents were absorbed on sheep erythrocytes before use. Immunodiffusion experiments were performed in 1% agarose, 3% polyethylene glycol gels.

RESULTS

Table I shows the agglutination titers of erythrocytes coated with various IgM proteins with the absorbed antiserum to protein Vin. Strong agglutination was obtained with erythrocytes coated with the homologous protein and a weaker one with protein Gen. No agglutination was obtained with 10 other IgM coats from patients with neurologic symptoms or 3 monoclonal IgM from patients with macroglobulinemia without neurologic symptoms. Erythrocytes coated with polyclonal IgM were not agglutinated.

Inhibition of the system with protein Vin as the coat and absorbed anti-Vin as the agglutinating serum was achieved only with the homologous protein down to a concentration of 8 μg/ml. Purified IgM or sera from patients with macroglobulinemia and neurologic symptoms (15 cases), or without neuropathy (15 cases) did not inhibit the reaction. Finally polyclonal IgM (5 mg/ml) or IgG (15 mg/ml) were not inhibitory.

Because it has previously been shown that coating erythrocytes with an IgM different from that used to prepare the anti-idiotypic serum offers a very sensitive system to reveal cross-idiotypic specificities among proteins with similar antibody activity (2, 13, 14), further experiments were performed with IgM Gen as the coat and the anti-Vin as the agglutinating antiserum (Table II). As expected, protein Vin and Gen were inhibitory down to a concentration of 15 μg/ml. Fab fragments but not the Fc fragment of protein Gen inhibited this system. Furthermore, 2 out of 10 IgM and 1 out of 4 sera from patients with macroglobulinemia and polyneuropathy inhibited this system at low concentrations (Tables II and III). Here again, Fab but not Fc fragments from IgM Ngu were inhibitory. It is of interest that serum Cla, which inhibited the system at high dilution was obtained from the sister of patient Vin.

One out of four sera from patients with monoclonal IgG or IgA and neuropathy inhibited the system. The isolated 7S fraction (but not the 19S fraction) from this serum contained the inhibitory activity, therefore confirming that the monoclonal IgGκ from patient Haa probably bore the cross-idiotypic antigenic determinants.

The cross-idiotypic system was not inhibited by 3 purified IgM and 70 sera from patients with WM without neurologic symptoms nor by polyclonal IgM (5 mg/ml) or polyclonal IgG except at high concentrations (15 mg/ml) (Tables II and III).

By precipitation the anti-Vin serum reacted with IgM from patients Vin, Gen, and Ngu. A partial identity was noted between the precipitin lines formed by protein Vin and IgM Gen or Ngu. The precipitin line of protein Ngu spurred over that of protein Gen. An additional macroglobulinemic serum (Rab) from a patient with neuropathy was positive in this system,

TABLE I

Agglutination of Sheep Erythrocytes Coated with Various IgM by Different Dilutions of the Absorbed Anti-Vin Antiserum

IgM coat	Dilution of antiserum							
	1/1	1/4	1/16	1/32	1/64	1/256	1/1,024	1/2,048
WM with neuropathy								
Vin	+++	+++	+++	+++	+++	+++	+	0
Gen	+++	++	+	Traces	0	0	0	0
Ngu	0	0	0	0	0	0	0	0
Can	0	0	0	0	0	0	0	0
Rab	0	0	0	0	0	0	0	0
Others (7 IgM)	0	0	0	0	0	0	0	0
WM (3 IgM)	0	0	0	0	0	0	0	0
Polyclonal IgM	0	0	0	0	0	0	0	0

TABLE II

*Hemagglutination Inhibition by Purified IgM and Fragments**

Inhibitor	Inhibitor concentration, *mg/ml*									
	2	1	0.5	0.25	0.125	0.06	0.03	0.015	0.007	0.003
WM with neuropathy										
Vin	0	0	0	0	0	0	0	Traces	+	++
Gen	0	0	0	0	0	0	0	0	+	++
Fab Gen	0	0	0	0	0	0	0	+	++	++
Fc Gen		tr	+	+	++	++	++	++	++	++
Ngu	0	0	0	0	0	0	0	+	++	++
Fab Ngu	0	0	0	0	0	Traces	+	++	++	++
Fc Ngu		tr	+	+	++	++	++	++	++	++
Can	0	0	0	0	0	+	+	++	++	++
Rab	+	+	++	++	++	++	++	++	++	++
Others (7 IgM)	+ or ++		+ or ++	++	++	++	++	++	++	++
WM (3 IgM)	+ or ++		+ or ++	++	++	++	++	++	++	++
Polyclonal IgM	+		++	++	++	++	++	++	++	++
Polyclonal IgG	+		++	++	++	++	++	++	++	++

* Anti-Ig Vin idiotypic serum/erythrocyte coat IgM Gen.

whereas it was unreactive by hemagglutination inhibition in the two systems tested. All other sera and purified IgM used were negative in precipitation tests.

DISCUSSION

Cross-idiotypic antigenic determinants were shared by 4 out of 12 purified IgM from patients with WM and polyneuropathy. This was best shown by hemagglutination and hemagglutination inhibition experiments using as erythrocyte coat a different IgM than the one used to rise the idiotypic antiserum. Indeed it had been shown in other systems (2) that this procedure was necessary to bring out cross-idiotypic reactivity between monoclonal Ig with similar antibody activity from unrelated individuals.

The reactivity was located within the Fab fragment of the IgM as shown by hemagglutination inhibition

TABLE III

*Hemagglutinin Inhibition by Sera from Patients and Controls**

Inhibitor	Serum level of monoclonal Ig	Serum dilution						
		1/1	1/4	1/16	1/64	1/128	1/256	1/512
	mg/ml							
WM with neuropathy								
Cla	7	0	0	0	0	0	0	+
Bec	4	++	++	++	++	++	++	++
Leb	20	+	++	++	++	++	++	++
Pec	20	0	0	+	++	++	++	++
Monoclonal IgG‡								
Haa	4	0	0	0	0	+	++	++
Men	6	+	++	++	++	++	++	++
Voi	10	Traces	Traces	+	++	++	++	++
Monoclonal IgA‡								
Mou	30	Traces	+	+	++	++	++	++
WM								
70 sera	7 to 40	Traces or +	+ or ++	++	++	++	++	++

* Anti-IgM Vin idiotypic serum/erythrocyte coat IgM Gen.

‡ Sera from patients with a monoclonal IgG and IgA and a neuropathy.

performed with IgM fragments from two of the cross-idiotypic IgM. Normal polyclonal IgM or IgG and monoclonal IgM from macroglobulinemic patients without neuropathy were unreactive except for a slight inhibition at high concentration of IgG. This latter finding suggests that cross-idiotypic antigens may be present on a very small number of normal IgG molecules as already found in other cross-idiotypic systems involving IgM with anti-IgG (2) or cold agglutinin (15) activities.

Hemagglutination inhibition was also tested with the whole serum from patients with monoclonal Ig with (8 sera) or without (70 sera) neurologic symptoms. No inhibition was observed with sera from the latter group. By contrast, two additional sera from patients with a neuropathy contained Ig with cross-reactive determinants. One was a monoclonal IgM and was obtained from the sister of the patient Vin whose IgM was used to raise the anti-idiotypic serum. The other serum was of special interest because the monoclonal Ig was of the IgG class. Isolation of the 7S fraction from this serum confirmed that the IgGκ was likely to possess the cross-idiotypic determinants. That cross-idiotypic antigens may be shared by antibodies of different classes has been already shown for IgM and IgA cold agglutinins as well as for IgM and IgG with rheumatoid factor specificity (15, 16). It must be noted that several idiotypic determinants are probably involved in our test system. Some antibodies reacted with antigenic determinants unique to the IgM Vin used for immunization, since the hemagglutinating system using the anti-Vin serum and erythrocytes coated with protein Vin could be inhibited only with the homologous IgM. The amount of protein needed to inhibit the cross-idiotypic system using anti Vin serum and erythrocyte coat Gen differed from one inhibitor to another. The presence of different cross-idiotypic determinants was confirmed by gel precipitation because only a partial identity was observed between the three IgM proteins that gave a precipitin line with the absorbed anti-Vin antiserum. The other cross-idiotypic proteins (protein Can, Haa, and Cla) defined by hemagglutination inhibition did not precipitate with anti-Vin antiserum. By contrast, an additional IgM (Rab) from a patient with WM and neuropathy gave a reaction of partial identity with IgM Vin by precipitation, whereas it was negative by hemagglutination inhibition. It may therefore indicate the existence of another cross-idiotypic system different from the one described here.

In two other systems, (IgM with anti-IgG or anti-erythrocyte antibody activity) the cross idiotypicity was clearly related to the combining site of the molecules because the monoclonal IgM no longer reacted with the anti-idiotypic serum when combined with their respective antigens (1, 2). Furthermore, structural studies of the cross-idiotypic proteins in the anti-IgG cross-idiotypic system showed that the heavy chains of the IgM belonging to the same cross-idiotypic group, not only belonged to the same V_H subgroup but also showed marked sequence similarities in the first and second hypervariable regions, which are thought to be directly involved in antigen binding (17). Therefore our finding of cross-idiotypic specificities among some monoclonal IgM from patients with WM and polyneuropathy may suggest that these IgM share a similar antibody activity, which may be directed against the nerve or some components involved in the pathogenesis of the polyneuropathy. However, the corresponding antigen is presently unknown and the direct proof of such an antibody activity is therefore lacking. The recent availability of purified antigens from neurological tissues and a study of the binding of the monoclonal IgM to nerve structures should allow to test this hypothesis. In this respect it is of interest that Ig deposits have been documented on nerve biopsies from patients with WM and neuropathy (5, 10). Such deposits may be related to an antibody activity of the IgM. One should be cautious however in the interpretation of these data because Fc or complement receptors have been found on nerve (18, 19) and may mediate a nonspecific trapping of Ig or immune complexes.

It should also be stressed that antibodies only rarely mediate nerve injuries in experimental models (20) and that their role in human demyelinating diseases is still controversial (21). Several mechanisms, apart from an antibody activity of the monoclonal IgM, may cause the neurologic symptoms observed in patients with WM: hyperviscosity, bleeding tendency, lymphoid infiltrates, and amyloidosis (4, 22). In this context it should be stressed that in our experience, chemotherapy and/or plasmapheresis are successful in less than one-third of the patients with WM and neuropathy and it may well turn out that the effectiveness of the treatment is related to the mechanisms involved in nerve injury.

Those patients whose IgM possess idiotypic determinants such as those described in the present paper may well represent a subgroup in which plasmapheresis might be helpful. Our findings may therefore have therapeutic implications in the future.

Note added in proof. Since this manuscript was submitted, additional experiments using an idiotypic antiserum to the protein Rab showed the existence of a second cross-idiotypic system involving protein Rab and Vin as suggested in the discussion of this paper.

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