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Plasma cell dyscrasia and peripheral neuropathy: Identification of the myelin antigens that react with human paraproteins

(peripheral nerve myelin/myelin proteins/monoclonal antibodies)

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ABSTRACT **In some cases of polyneuropathy and plasma cell dyscrasia, the monoclonal antibodies react with human peripheral nerve myelin. To identify the myelin antigens involved, we separated the proteins of human central and peripheral nerve myelin by polyacrylamide gel electrophoresis, transferred the proteins onto nitrocellulose sheets, and used an immunoenzymatic technique to detect the reactive antigens. Serum IgM but not IgG from three patients with neuropathy and complement-fixing anti-human myelin IgM paraproteins immunostained a protein of approximately 100,000 daltons in human peripheral nerve myelin and a protein or closely migrating proteins of similar size in human central nervous system myelin. In a fourth patient, both IgM and IgG immunostained the antigen. Immunostaining was specific for the paraprotein light chain type, and absorption of the patients' sera with human peripheral nerve myelin eliminated the reaction with the central nervous system proteins. No reaction was seen with rabbit peripheral nerve myelin or with membranes prepared from human myotubes, human T cells, or human fibroblasts. Control sera from six patients with neuropathy and IgM paraproteins that did not react with myelin, from four patients with IgM paraproteins but no neuropathy, and from three normal subjects did not immunostain myelin.**

Monoclonal antibodies reactive with autoantigens have been described (1, 2) in a number of patients with paraproteinemia or plasma cell dyscrasia. Peripheral neuropathy has been associated with a number of paraproteinemic syndromes (3–8), and in some of these patients the monoclonal paraproteins were found to react with human peripheral nerve myelin (9, 10), but the identity of the specific myelin antigens involved is not known.

Here we report that, in four patients with IgM paraproteins and peripheral neuropathy, the IgM antibodies reacted with a protein of approximately 100,000 daltons in human peripheral nerve myelin (PNM) and with a protein or proteins of similar molecular weight in human central nervous system myelin (CNM).

METHODS

Patients. Serum was obtained from four patients who had plasma cell dyscrasia and peripheral neuropathy and whose IgM paraproteins reacted with human PNM in the complement-fixation and immunoabsorption assays (9, 10). For controls we used serum from six patients who had neuropathy but whose IgM paraproteins did not react with human PNM in the complement-fixation or immunoabsorption assay, from four patients with IgM paraproteins but no neuropathy, and from three normal subjects.

Preparation of Membranes and Polyacrylamide Gel Electrophoresis. Human brain white matter and sciatic and femoral nerves were obtained at autopsy (12–18 hr after death) from patients who had no neurological disease, and CNM and PNM were isolated by sucrose density ultracentrifugation (11, 12). Three different PNM and CNM preparations were used. PNM was also prepared from baboon or rabbit nerve, and membranes were prepared from T cells isolated from a patient with T-cell leukemia and from cultured human myotubes and fibroblasts from normal individuals (provided by Armond Miranda). The membrane fractions were prepared by sonication, centrifugation at 1000 × *g* for 10 min, and centrifugation at 100,000 × *g* for 1 hr to pellet the membranes. The myelin and other membrane fractions were lyophilized and delipidated by three washes in cold ether ethanol, 3:2 (vol/vol). The proteins were solubilized in sample buffer and separated by electrophoresis in 12% polyacrylamide slab gels with a 6% stacking gel in sodium dodecyl sulfate (13).

Immunoenzymatic Identification of the Antigens. After electrophoresis the proteins were transferred onto nitrocellulose sheets (Schleicher & Schuell) and the antigens that reacted with the paraproteins were identified by an immunoenzymatic technique (14). The nitrocellulose blots were incubated with 3% bovine serum albumin to saturate the remaining protein binding sites, treated with serum from a patient or control, washed, and treated with peroxidase-conjugated rabbit anti-human IgM (αIgM-P), peroxidase-conjugated anti-human IgG (αIgG-P), or peroxidase-conjugated anti-human κ ($\alpha\kappa$-P) or λ ($\alpha\lambda$-P) light chains, at a dilution of 1:500 (Dako, Accurate Chemical, New York). The immune reactions were visualized with 0.1% 3,3'-diaminobenzidine tetrahydrochloride (Sigma)/0.1% imidazole/0.1% hydrogen peroxide.

Serum was screened for the presence of IgM reactive with human CNM or PNM proteins by incubating the nitrocellulose blots with serum at a dilution of 1:200, followed by αIgM-P as the second antibody. Reactive serum was also examined by using αIgG-P, $\alpha\kappa$-P, or $\alpha\lambda$-P as the second antibody. Reactive sera from two patients were absorbed with human PNM by incubating 0.1 ml of 1:10 dilution of serum with 1 mg of lyophilized PNM overnight, five times, and the absorbed sera then were used to immunostain PNM and CNM. Control sera were absorbed with equal amounts of rat liver powder. The nitrocellulose blots were also immunostained with rabbit antiserum to mouse myelin basic protein or rabbit antiserum to cerebro-

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Abbreviations: PNM, peripheral nerve myelin; CNM central nervous system myelin; αIgM-P, peroxidase-conjugated rabbit anti-human IgM; αIgG-P, peroxidase-conjugated rabbit anti-human IgG; $\alpha\kappa$-P, peroxidase-conjugated anti-human κ light chains; $\alpha\lambda$-P, peroxidase-conjugated rabbit anti-human λ light chains.

sides (gift of M. Rapport) at a dilution of 1:200 and counterstained with peroxidase-conjugated goat anti-rabbit immunoglobulins at a dilution of 1:500. Sera that reacted with human myelin also were tested for reactivity against rabbit or baboon PNM and against membranes of myotubes, T cells, and fibroblasts. Relative mobilities of the proteins were compared to standard proteins run in parallel and the molecular weights were calculated (15).

RESULTS

The electrophoretic separation of CNM and PNM proteins is shown in Fig. 1. Peripheral nerve P1 protein and CNM basic protein are antigenically identical (16) and antibodies to basic protein immunostained both (Fig. 2*A*). When human serum was used as the first antibody and αIgM-P as the second, serum from each of the four patients with complement-fixing anti-PNM IgM paraproteins immunostained a protein with the same mobility in PNM and one or several closely migrating proteins in CNM (Fig. 2*B*). The PNM protein migrated with an apparent molecular weight of approximately 100,000 whereas the CNM antigen ran as a broad band or as closely migrating multiple bands of similar molecular weight. No reaction was seen with serum from the six patients with neuropathy and IgM paraproteins that did not have complement-fixing anti-PNM activity, from the four patients with IgM paraproteins but no neuropathy, or from the normal subjects. Immunostaining with αIgM-P alone also showed no reaction (Fig. 2 *C* and *D*).

To determine whether the patients' sera contained IgG antibodies directed at the same myelin antigens, immunostaining was performed with αIgG-P as the second antibody. Immunostaining of PNM was impossible to interpret because incubation with αIgG-P alone yielded multiple bands (Fig. 3*D*),

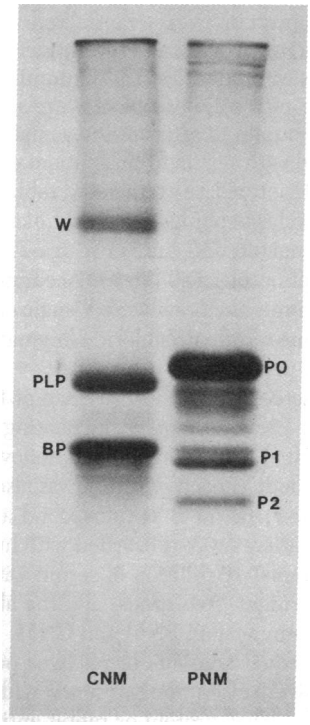

FIG. 1. Polyacrylamide gel electrophoresis of human myelin proteins. The major proteins in CNM are the Wolfgram protein (W), proteolipid protein (PLP), and basic protein (BP). In PNM they are the P0 glycoprotein, the P1 basic protein, and the P2 basic protein (9). Note that the P1 protein migrates as a doublet. The gel is stained with Coomassie blue.

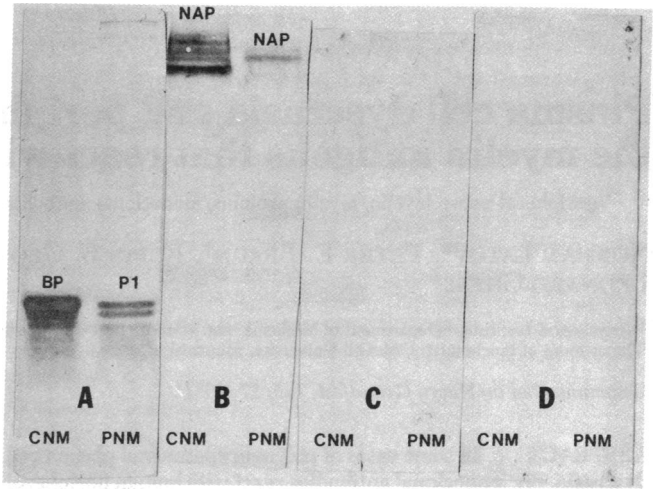

FIG. 2. Immunostaining of CNM and PNM proteins after electrophoresis and transfer onto nitrocellulose blots. (*A*) Anti-basic protein antiserum; (*B*) serum from a patient with neuropathy and anti-PNM IgM paraprotein counterstained with αIgM-P; (*C*) serum from a patient with neuropathy and IgM paraprotein but without anti-PNM activity counterstained with αIgM-P; (*D*) normal serum counterstained with αIgM-P. Note that the serum in *B* immunostained the neuropathy-associated proteins (NAP) in CNM and PNM. BP, basic protein; P1, P1 protein.

possibly because of adsorption of IgG to PNM during homogenization (17, 18) or because of cross-reactive antigens in PNM. However, αIgG-P did not react with CNM, and immunostaining CNM with the patients' sera and αIgG-P revealed no reaction in three of the patients (Fig. 3*B*). In one of these four patients, immunostaining with αIgG-P was identical to that seen with αIgM-P (Fig. 3*C*). Immunostaining with the paraproteins and ακ-P or αλ-P showed strong reactivity when the second antibody corresponded to the paraprotein light chain type but no or very weak reactivity when the other light chain reagent was used. Absorption of the patients' sera with human PNM eliminated the immunostaining of both human PNM and CNM, but absorption with rat liver powder did not. No reaction was seen when antiserum to cerebrosides was used, and the

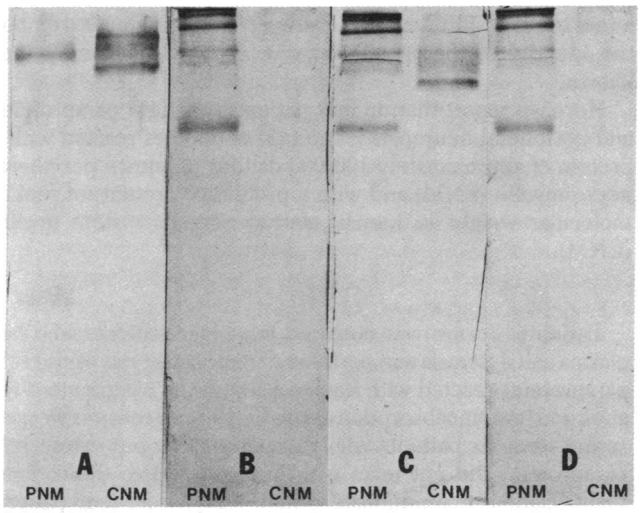

FIG. 3. Immunostaining of CNM and PNM after electrophoresis and transfer onto nitrocellulose blots. (*A*) Representative of serum from any of the four patients with anti-PNM IgM antibodies counterstained with αIgM-P; (*B*) representative of serum from three of the four patients counterstained with αIgG-P; (*C*) serum from one of the four patients counterstained with αIgG-P; (*D*) αIgG-P alone.

patients' sera did not immunostain proteins of rabbit PNM or of membranes from human T cells, myotubes, or fibroblasts (Fig. 4). Immunostaining of baboon myelin, however, was identical to that of human myelin.

DISCUSSION

In the four patients with neuropathy and anti-PNM IgM paraproteins, the IgM antibodies reacted with a protein of approximately 100,000 daltons in human PNM and with one or more proteins of similar molecular weight in human CNM. The multiple closely spaced bands in the CNM were seen with three different CNM preparations and probably resulted from postmortem autolysis of a single protein because a single dense band was frequently seen and because absorption with human PNM eliminated the immunostaining of all the CNM bands. The identified antigens are significantly larger than the major myelin proteins but are similar in size to myelin-associated glycoprotein (19). The antigen may be species restricted because it is found in human and baboon PNM but not in rabbit PNM by a number of assays including complement fixation, immunoabsorption, and immunostaining of myelin proteins (9, 10). We therefore refer to these proteins as the CNM or PNM "neuropathy associated proteins" (NAP).

In serum from three of the four patients there was immunostaining of the CNM neuropathy-associated proteins by IgM but not by IgG, but in one patient both IgG and IgM immunostained them. Immunostaining by the patients' serum was specific for the paraprotein light chain type, indicating that the antimyelin antibodies were monoclonal. Multiple M-components and the presence of immunoglobulins with the same idiotype but of a different heavy chain class than in the paraprotein have been reported by others (20, 21), and switching from synthesis of IgM to synthesis of IgG, which normally occurs during maturation, may also occur in malignant lymphocytes (22).

Although the paraproteins immunostained both CNM and PNM proteins, these patients had a demyelinating peripheral neuropathy rather than a cerebral disorder. This could be explained if the immunoglobulin concentration in brain were kept low by the blood–brain barrier or if the CNM antigenic site were not exposed *in situ*.

It has been reported that monoclonal IgM antibodies from 5 of 16 patients with macroglobulinemia and polyneuropathy shared idiotypic antigenic determinants, suggesting that some nerve antigens may be involved more frequently than others (23). In animals, immunization with P0 glycoprotein, P2 basic protein, or galactocerebroside may induce an experimental allergic neuritis (24–26), but none of these has been shown to be immunologically involved in humans (27). It is unlikely that the paraproteins share antigenic specificity with anticerebroside antibodies because there was no immunostaining of neuropathy-associated proteins by anticerebroside antiserum and because the paraproteins did not react with rabbit PNM or with chloroform/methanol-treated human PNM (9, 10) which contain cerebrosides. Perhaps only a few myelin constituents can induce autoimmunity in humans, and the proteins we identified may be involved in other autoimmune demyelinating diseases of the peripheral or central nervous system. The normal role of these proteins remains to be elucidated.

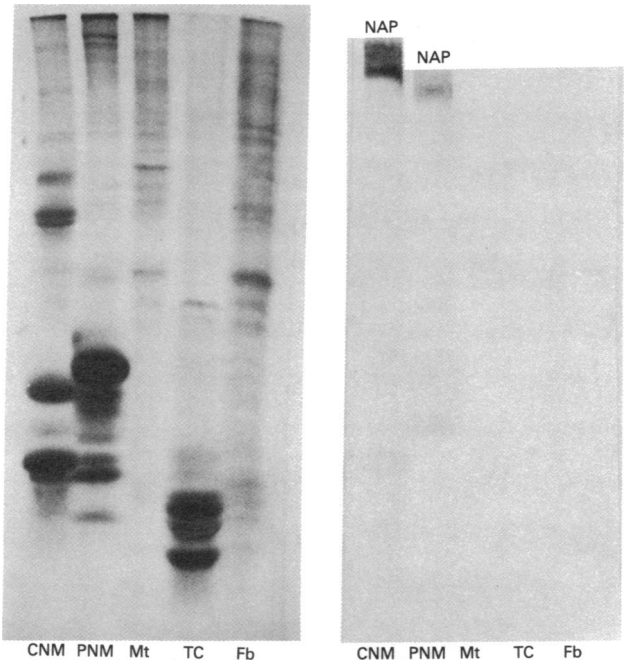

FIG. 4. (*A*) $NaDodSO_4$/polyacrylamide gel electrophoresis of human CNM, human PNM, human myotube membrane (Mt), human T-cell membrane (TC), and human fibroblast membrane (Fb). Proteins were stained with Coomasie blue. (*B*) Immunostaining of the membrane proteins in *A* after transfer onto nitrocellulose. Serum from a patient with anti-PNM IgM antibodies was counterstained with αIgM-P.

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