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Production and Characterization of Monoclonal Antibodies to the Extracellular Domain of P0

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Seven monoclonal antibodies were raised against the immunoglobulin-like extracellular domain of P0 (P0-ED), the major protein of peripheral nervous system myelin. Mice were immunized with purified recombinant rat P0-ED. After fusion, 7 clones (P01–P07) recognizing either recombinant, rat, mouse, or human P0-ED were selected by ELISA and were characterized by Western blot, immunohistochemistry, and a competition assay. Antibodies belonged to the IgG or IgM class, and P04–P07, reacted with P0 in fresh-frozen and paraffin-embedded sections of human or rat peripheral nerve, but not with myelin proteins of the central nervous system of either species. Epitope specificity of the antibodies was determined by a competition enzyme-linked immunosorbent assay (ELISA) and a direct ELISA using short synthetic peptides spanning the entire extracellular domain of P0. These assays showed that P01 and P02 exhibiting the same reaction pattern in Western blot and immunohistochemistry reacted with different distant epitopes of P0. Furthermore, the monoclonal antibodies P05 and P06 recognized 2 different epitopes in close proximity within the neuritogenic extracellular sequence of P0. This panel of monoclonal antibodies, each binding to a different epitope of the extracellular domain of P0, will be useful for in vitro and in vivo studies designed to explore the role of P0 during myelination and in demyelinating diseases of the peripheral nervous system.

Key words: peripheral nervous system, myelin, epitope specificity, demyelination

INTRODUCTION

The glycoprotein P0 accounts for over 50% of the protein present in purified peripheral myelin (Greenfield et al., 1973; Ishaque et al., 1980). P0 is a member of the immunoglobulin superfamily containing one V-like immunoglobulin domain (Williams and Barcley, 1988) and has an apparent molecular mass of 28–30 kD. It is produced exclusively by myelinating Schwann cells (Trapp et al., 1987; Martini et al., 1988).

Recently it has been shown that the extracellular domain of P0 (P0-ED) acts as a homotypic adhesion molecule and has neurite outgrowth-promoting properties (Filbin et al., 1990; Schneider-Schaulies et al., 1990; D'Urso et al., 1990; Filbin and Tennekoon, 1991). Because of these properties P0 could be important for the initiation of myelination and the maintenance of compact myelin in the peripheral nervous system (Lemke et al., 1988).

Furthermore, there is evidence to suggest that P0 may be involved in the pathogenesis of demyelinating diseases of the PNS: (1) In some demyelinating polyneuropathies associated with a monoclonal gammopathy, high titers of anti-P0 antibody are found which recognize mainly carbohydrate determinants on P0 (Bollensen et al., 1987; Burger et al., 1990). (2) Antibodies to P0 can produce demyelination when injected into rat sciatic nerves (Hughes et al., 1985; Hosokawa et al., 1990). However, direct experimental evidence for a demyelinating role of antibodies to P0 in human neuropathies is still lacking (for review see Hartung et al., 1993). (3) P0 has also been described to induce experimental autoimmune ("allergic") neuritis (EAN) in rats (Milner et al., 1987). Recent experiments with the adoptive transfer model of EAN showed that one neuritogenic T-cell epitope is located within the amino acid sequence 56–71 of the extracellular domain of P0 (Linington et al., 1992). (4) In some patients with GBS, T cells reactive with human P0

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have been identified early in the disease course (Khalili-Shirazi et al., 1992).

Monoclonal antibodies recognizing extracellular epitopes with known amino acid sequences of P0 would be useful tools for in-vivo immunomodulation experiments and may thus help to define the precise role of P0 during demyelinating diseases. Therefore, we used the recombinant extracellular domain of P0 to establish a panel of monoclonal antibodies recognizing rat and human P0.

MATERIALS AND METHODS

Expression and Purification of Recombinant P0-ED

The P0-ED construct contained the 124 amino acid residues (amino acids 1–124) from the extracellular domain of rat P0 and a total of 20 amino acid residues from the pET3 expression vector system (Rosenberg et al., 1987). Expression of the 17.1 kD recombinant Ig-like P0-ED was carried out with as previously described (Schneider-Schaulies et al., 1990).

In brief, 100 ml of an overnight culture of bacteria [BL21(DE3)] containing plasmid pET3-P0-ED were added to 900 ml Luria-broth medium (Gibco, Karlsruhe, Germany) and were induced at 37°C with 1 mM isopropylthio-β-D-galactoside (IPTG) for 6 hr to express high levels of recombinant P0-ED. P0-ED expressing bacterial cultures (1,000 ml) were centrifuged at 4°C and 3,000 g for 20 min and lysed by 4 cycles of freeze-thawing (liquid nitrogen/37°C) and centrifuged at 32,000 g for 30 min to isolate the insoluble P0-ED in the form of inclusion bodies. The insoluble bacterial pellet was incubated twice overnight at 4°C with 25 ml extraction buffer (4 M urea, 50 mM Tris-HCl, pH 8.0). A crude lysate from 1,000 ml of a bacterial culture contained approximately 10 mg P0-ED protein as determined by SDS-PAGE. To purify P0-ED, the protein extract was size-fractioned twice on a Sephadex G-50 column using 4 M urea in Tris-HCl pH 8.0 as a running buffer.

The P0-ED containing fractions were collected and concentrated by pressure filtration through Amicon filters (PM 10, Witten, Germany) to give a final protein concentration of 2.5 mg/ml. The fraction was denatured at room temperature with dithioerythritol (DTE, 50 mg/ml), 6 M guanidinium · HCl, 100 mM Tris-HCl, pH 8.0, and reoxidized for 24 hr by 5 mM oxidized glutathione (Calbiochem, Bad Soden, Germany) in 100-fold excess volume to allow refolding of the molecule. After this renaturation procedure, P0-ED remained soluble in physiological buffers. SDS-PAGE using 15% acrylamide slab gels revealed 1 major band at 17 kD.

Immunization and Fusion

Balb/C mice (female, 6 weeks old, obtained from Zentralinstitut für Versuchstierzucht, Hannover, Germany) were primed with an intraperitoneal injection of 50 μg P0-ED in complete Freund's adjuvant (Difco, Detroit, Michigan) and boosted 5 times, at an interval of 6–8 weeks each, with 20 μg P0-ED in incomplete Freund's adjuvant until serum titers reached levels greater 1:50,000 in the enzyme-linked immunosorbent assay (ELISA). The Balb/C mice, known to be resistant to neuritogenic antigens, did not develop clinical signs of acute experimental autoimmune neuritis after immunization with P0-ED (data not shown).

Three days after the last injection, spleen cells were fused with the mouse myeloma cell line Ag8-653 obtained from the American Type Tissue Collection (Rockville, MD). The fused cells were distributed over ten 96-well microtiter plates (Nunc, Wiesbaden, Germany). Hybridoma cells were grown using standard methods, and supernatants were screened for the presence of antibodies directed against P0-ED by ELISA as described below. Hybridoma cells selected on the basis of the results in this ELISA were cloned by limiting dilution and direct picking of the clones from the plates.

ELISA

Ninety-six-well microtiter plates were coated with 4 μg/ml P0-ED in 0.1 M $NaHCO_3$ pH 9.6 at 4°C overnight and blocked with 0.5% bovine serum albumin (BSA) in phosphate-buffered saline (PBS). Plates were washed several times with 200 μl/well PBS, containing 0.1% Tween 20, and incubated with hybridoma supernatant for 30 min on a rotary shaker at 300 rpm. After a second washing, plates were incubated for 30 min with horseradish peroxidase-coupled goat-anti-mouse immunoglobulin (Boehringer, Mannheim, Germany; diluted 1:500 in blocking buffer). Antibody binding was visualized by using 2 mM ABTS [2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid); Boehringer, Mannheim] in 0.1 M acetate buffer, pH 4.2, and 2.5 mM hydrogen peroxide. Optical density (O.D.) was recorded at wavelengths of 405 and 495 nm with an ELISA reader.

Positive supernatants were also tested on reactivity to rat P0 measured in an direct ELISA. For this purpose 20 μg of rat myelin (vide infra) were included in the coating buffer containing 0.2% SDS. The plates were then processed as described above for P0-ED.

Competition and Direct ELISA With Synthetic Peptides

To investigate whether the monoclonal antibodies bound to independent epitopes of the extracellular domain of P0, the method of Friguet et al. (1983) was used in the ELISA system described above. Since only immunoglobulins of the same class could be tested with this method, P03 (IgG3) and P07 (IgG1) had to be excluded from this assay. Only IgMs with the same reaction pat-

terns in Western blot and immunohistochemistry were included. In brief, 2 different monoclonal antibodies of the same Ig subclass were added, either together or alone, into each well of microtiter plates coated with a relatively low amount of antigen (0.1 μg P0-ED/ml, 50 μl/well).

For this purpose culture supernatants of both tested antibodies were diluted with medium until each supernatant gave a similar absorbance in the P0-ED ELISA (described above) but were still concentrated enough to saturate the low amounts of coated P0-ED, as estimated by using different concentrations of the diluted supernatant. This resulted in nearly complete saturation of the epitope recognized by each antibody. If 2 antibodies were added together into one well, then after development with ABTS (see above), binding of antibodies to different epitopes yielded an increase of the measured O.D. at 405 nm compared to the values detected if only one antibody was added to the well.

Binding to the same or a nearby epitope did not result in an increased O.D. value if compared to the individual values. Similarly, the amount of antibody bound was assumed to be proportional to the measured O.D. at 405 nm. Competition was estimated by the additivity of the absorbance values as described elsewhere (Friguet et al., 1983).

Furthermore, the supernatants were tested by a direct ELISA (Linington et al., 1992) for their reactivity with small synthetic peptide sequences spanning the entire extracellular domain of P0: peptide 56–71 from rat P0 and peptides 1–20, 19–38, 38–56, 70–85, 84–110, 98–116, and 115–126 from bovine P0. The numbering sequence refers to the mature protein sequence.

Isotype Determination of Monoclonal Antibodies

Antibody isotypes were determined by ELISA using Ig-subclass-specific polyclonal rabbit-anti-mouse antibodies followed by goat-anti-rabbit Ig antibodies conjugated to horseradish peroxidase (Biorad, Munich, Germany).

Preparation of Myelin From Sciatic Nerves

Myelin from human, rat, mouse, and bovine PNS and CNS was prepared according to the method of Uyemura et al. (1972). Fresh bovine nervous tissue was obtained from the local slaughterhouse and human sciatic nerves from autopsies carried out at the Department of Pathology, University of Würzburg.

SDS-PAGE and Western Blot Analysis

SDS-PAGE was carried out on 10% and 15% acrylamide containing gels according to the method of Laemmli (1970). Proteins were stained with Coomassie blue. Western blot analysis was performed according to the method of Towbin et al. (1979), with minor modifications. Then 2 μg of P0-ED or 5 μg of myelin (human, rat, mouse, bovine) were loaded on to each lane. After SDS-PAGE, proteins were transferred electrophoretically to nitrocellulose membranes (Schleicher und Schüll, Dassel, Germany) at 80 mA for 2 hr. Unreactive binding sites were saturated for 2 hr with 30% FCS (Biochrom, Berlin, Germany) and 3% BSA. The nitrocellulose strips were then washed with PBS, pH 8.0, and 0.1% Tween, and incubated in sequence with hybridoma culture supernatants overnight at 4°C and, after washing, with alkaline-phosphatase coupled rabbit-anti-mouse Ig (Boehringer, Mannheim, Germany, diluted 1: 500) for another 60 min.

Nitrocellulose membranes were stained with NBT (para-nitrotetrazolium blue) and BCIP (5-bromo-4-chloro-3-indolylphosphate, Sigma, Munich, Germany), diluted in alkaline phosphatase buffer (100 mM Tris-HCl, 100 mM NaCl 5 mM MgCl, pH 9.4). Polyclonal rabbit-anti-P0-ED (final dilution 1:1,000) was used as a positive control and for internal standardization.

Immunohistochemistry

For immunohistochemical investigations, human and rat sciatic nerve and brain sections were studied using the streptavidin-biotin-peroxidase complex technique (Scheidt and Friede, 1987). Human and rat sciatic nerve were frozen without prior fixation in isopentane, cut into 10 μm sections on a Leitz cryostat, and air-dried for 45 min at room temperature. After fixation in acetone at −20°C for 10 min, the sections were blocked with 30% porcine serum (obtained from Sigma) to reduce nonspecific staining and were incubated overnight at 4°C with undiluted hybridoma culture supernatant. Sections were washed, incubated with 0.5% hydrogen peroxide in methanol 15 min at room temperature to block endogenous peroxidase activity, and then the biotinylated second antibody [sheep-anti-mouse immunoglobulin, $F(ab')_2$ fragments] preabsorbed with rat serum was applied for 30 min. Finally the peroxidase reaction product was developed with 0.05% diaminobenzidine and 0.01% hydrogen peroxide in PBS for 10 min.

For paraffin embedding tissue was fixed overnight in 4% paraformaldehyde as previously described (Jung et al., 1992). Staining on paraffin-embedded sections of 5 μm was performed as described above. In order to demonstrate the specificity of the staining, monoclonal antibodies were absorbed with a 10-fold excess of P0-ED (on a molar basis) for 30 min at 37°C before staining.

RESULTS

Selection of Monoclonal Antibodies

Selection of suitable hybridoma clones was done by using the ELISA technique. All the supernatants were

TABLE I. Summary of Reaction Pattern of P01–P07 in Western Blot and Immunohistochemistry

		Western blot			Histochemistry	
Antibody	Ig class	P0-ED	P0-rat	P0-human	Rat	Human
P01	IgM	+	−	−	−	−
P02	IgM	+	−	−	−	−
P03	IgG3	+	−	−	−	−
P04	IgM	+	+	−	+	−
P05	IgM	+	+	+	+	+
P06	IgM	+	+	+	+	+
P07	IgG1	+	+	+	+	+

All antibodies were tested for their reactivity with recombinant P0-ED, and human and rat P0, with Western blot and by immunohistochemistry. The antibodies P01–P07 all reacted with P0-ED, but only 4 of them (P04–P07) recognized human or rat P0. Antibodies with the same reaction pattern were further characterized in a competition and direct ELISA using synthetic peptides.

tested for reactivity with P0-ED in the ELISA. From the first fusion, 16 hybridoma supernatants were obtained that gave a positive result in this test. These selected supernatants were further evaluated for their reactivity with whole-rat myelin in the ELISA. Only 4 of the 16 selected antibodies gave a positive signal, indicating that they not only recognize recombinant P0-ED but also native P0 of the rat. These 4 monoclonal antibodies were designated P04–P07, and 3 of the remaining group giving rise to the highest absorbance values in the ELISA with P0-ED were designated P01–P03. The 7 monoclonal antibodies were then further characterized with Western blot, competition, and direct ELISA, and by immunohistochemistry. The other 9 antibodies were not studied further.

Antigenic Specificity and Species Distribution of P01–P07

Antigen specificity and species distribution of P01–P07 were analysed by Western blot. The results are summarized in Table I. All of the 7 selected antibodies recognized a single band with an apparent molecular mass of approximately 17 kD in the Western blot, corresponding to P0-ED (Fig. 1, lanes 1 and 4).

When rat and human myelin proteins were blotted, supernatants P01–P03 failed to recognize P0. Only P05–P07 reacted with whole native rat and human P0 banding at 29 kD (Fig. 1), thus confirming the results obtained in the ELISA using myelin. Interestingly, P04 only reacted with rat P0 but not with human P0 (Fig. 1). P04–P07 also recognized mouse P0 but failed to react with bovine P0 in the Western blot (data not shown). No other peripheral myelin proteins, including MBP or P2, were detected with these antibodies, nor did they recognize myelin proteins from the rat or human central nervous system in the Western blot, thus establishing their specificity for P0.

In most myelin preparations, one band with a molecular weight of 29 kD was exclusively detected. In a few myelin preparations, a further band at 24 kD was detected, probably representing a degradation product of P0. Its appearance was variable and not species dependent. This observation may be attributed to the presence of proteases in the myelin preparation (see below).

Immunocytochemistry

Immunohistochemical studies on frozen and paraffin-embedded sections of adult human and rat nervous tissue revealed that the antibodies P04–P07 bound specifically to human or rat peripheral nerve myelin (Table I). In the paraffin-embedded sections the immunoreactivity was exclusively localized in compacted myelin sheaths, whereas the axonal cytoplasm and capillary endothelial cells were completely spared (Fig. 2).

Staining of frozen sections revealed the same reaction pattern of antibodies P04–P07 as in the paraffin sections. However, in the frozen sections staining was less intense and background staining was higher, resulting in less contrast when compared to the paraffin sections (data not shown). Preabsorption of the monoclonal antibodies with P0-ED resulted in complete loss of staining, indicating that staining of P0 was specific. No staining was observed in sections of human and rat brain (data not shown).

Epitope Specificity of P01–P07

P01–P03 and P05–P07 antibodies exhibited a similar reaction pattern in the ELISA, Western blot, and by immunohistochemistry (Table I). To investigate whether these monoclonal antibodies were reactive to the same or to independent epitopes, competition and direct ELISAs were performed.

The results obtained with the competition ELISA are summarized in Table II. The theoretical sum of reactivities of P01 and P02 at saturation levels is 0.74, which is reached approximately by the experimental

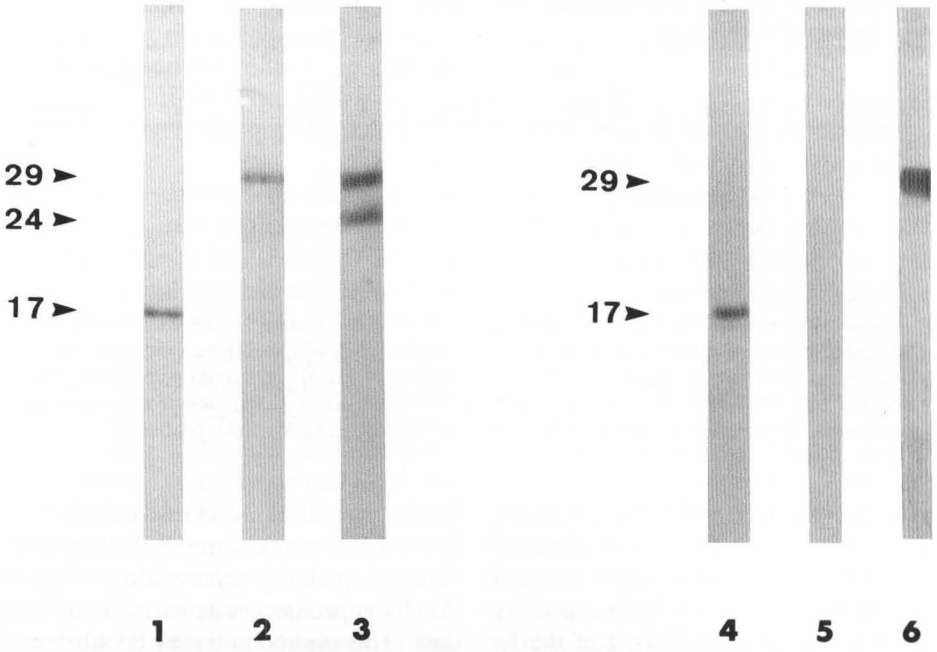

Fig. 1. Immunostaining of peripheral nerve myelin and P0-ED by the monoclonal antibodies P07 (**lanes 1–3**) and P04 (**lanes 4–6**) in Western blots. Lanes 1 and 4 correspond to P0-ED, lanes 2 and 5 correspond to human myelin, and lanes 3 and 6 correspond to rat myelin. Note the presence of a 24-kD P0 band, possibly a cleavage product, in the rat myelin preparation. Arrows on the left indicate the molecular mass in kilodaltons.

value of 0.64 and indicates that each antibody binds to a different and distant epitope. The values of absorbance obtained with P05 and P06 when added together (0.21) were significantly below their singular values (0.59 and 0.41), indicating that both antibodies bind to epitopes in close vicinity and that the large IgM molecules exert steric hinderance on each other.

When tested in a direct ELISA only 2 of the antibodies, P05 and P06, were found to react with any of the panel of overlapping synthetic P0 peptides listed in the text previously. Interestingly, although both antibodies recognized an epitope within the same peptide p56–71, they differ markedly with respect to their binding relative to P0-ED (Table III). This confirmed the observation made in the competition ELISA test that P05 and P06 bind to 2 different nearby epitopes. The other 5 monoclonal antibodies, P01–P04 and P07, also reactive with whole P0-ED, displayed no reactivity to any of the tested sequences.

DISCUSSION

We have described and characterized 7 monoclonal antibodies, P01–P07, which recognize the extracellular domain of human, rat, and mouse P0. The antibodies appear to react with different epitopes of the protein backbone of P0, as concluded from their recognition patterns in Western blot, immunohistochemistry, and competition or direct ELISA with synthetic peptides.

P01 and P02 clearly react with different epitopes, as evidenced in the competition ELISA. P03 and P07 had to be excluded from the competition assay, as their Ig classes differ from the others. In the same assay addition of both P05 and P06 together showed a significant reduction of absorbance. Such a reduced value can be obtained with antibodies of the IgM class if both bind 2 epitopes in close proximity. The direct ELISA with these 2 antibodies confirmed that they bind to nearby epitopes: The binding of P05 and P06 to the peptide p56–71 differed considerably when compared to their ability to bind to P0-ED in a direct ELISA. This difference suggests that the 2 monoclonal antibodies recognize spatially close but dissimilar epitopes within the neuritogenic amino acid sequence 56–71 of the extracellular domain of P0. The low binding of P05 to the peptide may indicate that conformation of the epitope is altered in the peptide, relative to that in P0-ED, or alternatively, the interaction between the peptide and plastic surface of the ELISA plate interferes with antibody binding.

The other 5 monoclonal antibodies, P01–P04 and P07, failed to bind to any peptide sequence tested, probably because most of the sequences of P0 used were of bovine origin. The protein sequence of bovine P0-ED differs from human P0-ED in 7 and from rat P0-ED in 6

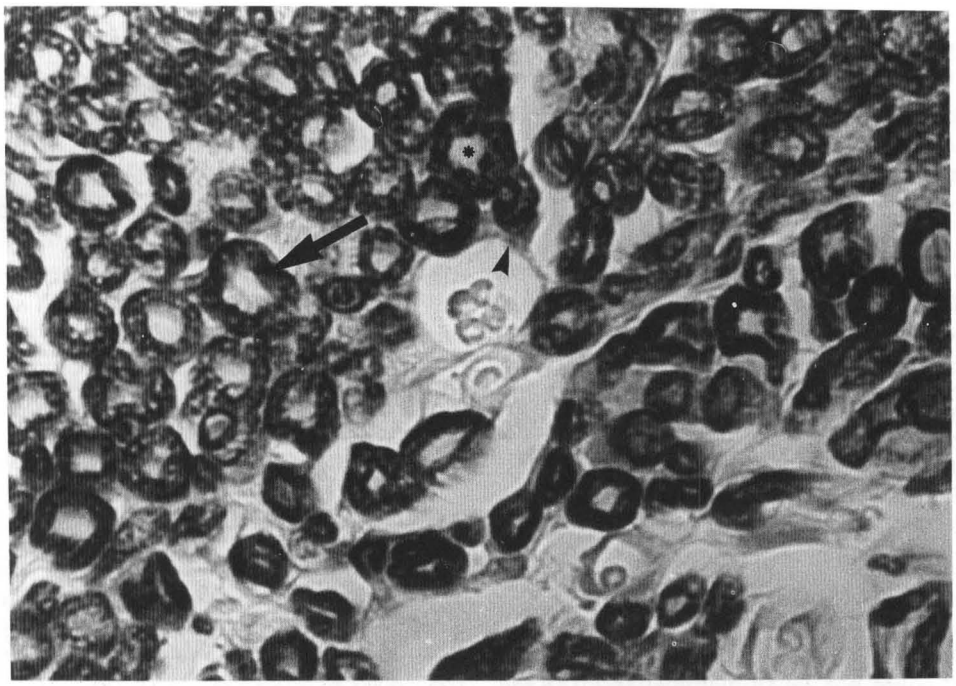

Fig. 2. Immunohistochemical characterization of P07 monoclonal antibody. Sciatic nerve from rat was fixed overnight in 4% paraformaldehyde, embedded in paraffin, and sections (5 μm) were stained by the streptavidin-biotin-peroxidase technique. Specific staining of myelin sheaths (arrow) of the peripheral nerves is seen. Axonal cytoplasma (asterisk) or capillary endothelial cells (arrowhead) are not labeled. Background staining is very low. Magnification: ×440.

TABLE II. Results of the Competition ELISA Test for the Monoclonal Antibodies P01, P02, P05, and P06

Anti-P0-ED-antibody	O.D. (405 nm)	Theoretical O.D.
P01	0.36 ± 0.02	—
P02	0.38 ± 0.05	—
P01 + P02	0.64 ± 0.02	0.74
P05	0.59 ± 0.04	—
P06	0.41 ± 0.02	—
P05 + P06	0.21 ± 0.03	1.00

Hybridoma supernatants of each antibody were combined as indicated and added to microtiter plates coated with P0-ED. The resulting binding was visualized (see Materials and Methods) and quantified by reading the optical density (O.D.) at 405 nm. Results are means ± standard deviation of the O.D. from 2 independent experiments done in quadruplicate. P03 and P07 were not included in this test because they were not of the IgM isotype.

TABLE III. Monoclonal Antibodies P05 and P06 Recognize the Neuritogenic Determinant of Rat P0

	Monoclonal antibody	
Coating antigen	P05	P06
P0-ED	1.75 ± 0.14	2.90 ± 0.16
p56-71	0.68 ± 0.07	0.44 ± 0.03
BSA	0.16 ± 0.09	0.21 ± 0.08
Ratio P0-ED/p56-71	3.06	11.69

Culture supernatants obtained from all 16 clones were tested by ELISA for reactivity with P0-ED and the synthetic P0 peptides listed in Materials and Methods. Only the monoclonal antibodies P05 and P06 exhibited reactivity with both P0-ED and a peptide, each recognizing an epitope within the amino acid sequence residues 56–71 of rat P0. However, binding to the peptide was considerably weaker than that observed for P0-ED, being most marked for P06. Results are expressed as the mean values ± standard deviation of optical density at 405 nm obtained from triplicate wells. These values are not corrected for the background against BSA, which is listed separately. The second peroxidase conjugated anti-mouse antibody did not bind to either P0-ED or any of the synthetic peptides.

amino acid residues (Lemke and Axel, 1985; Sakamoto et al., 1987; Ilayasaka et al., 1991). This explains the lack of recognition of the peptides used and of total bovine P0 in the Western blot.

Furthermore, it is well known that small peptides often differ from the whole protein in their 3-dimensional structure and, in consequence, also in their antigenicity. They sometimes even escape detection by specific antibodies in ELISA, as short epitopes may be masked by adherence to the ELISA plate. This last point is confirmed by the much lower O.D. values detected with P05 and P06 using peptide-coated plates, compared to the absorbance values recorded when total P0-ED is used at the same molar concentration (Table III).

P07 showed the same reaction pattern in Western blot and immunohistochemistry as P05 and P06, but it

was negative in the direct ELISA test, indicating binding to a different epitope. P04 must react with a different epitope than the other 6 antibodies, because it recognized only rat P0, but not human P0 in the Western blot.

In the Western blot of rat myelin, the antibodies occasionally recognized a protein with an apparent molecular mass of 24 kD, besides the native P0. This was assumed to be a P0 degradation product. Recently it has been shown that rat P0 is cleaved by proteases present in myelin of the peripheral nervous system, resulting in a 24 kD P0 molecule (Agrawal et al., 1990; Van den Berg et al., 1990). Thus the protein stained in some myelin preparations in our experiments could be such a cleavage product. In conclusion, we show that the selected antibodies react with different epitopes of P0-ED, although only the epitopes recognized by P05 and P06 could be defined in terms of amino acid sequence.

The other 9 antibodies selected initially by ELISA were not further characterized in this study, because they gave a positive result with P0-ED in the ELISA and Western blot but could not detect mammalian P0 in the tests used. Therefore they either recognize the pET-derived sequences of P0-ED, or P0 epitopes which are not accessible to them in the native P0 molecule. These conformational differences of P0-ED and native P0 could be due to the lack of carbohydrate moieties on the recombinant form of P0.

In the past, several groups have produced antibodies to P0 (Franko et al. 1982; Miller et al. 1984; Bollensen et al., 1990). Antibodies 592 and 593 (a generous gift from Dr. M. Schachner, Department of Neurobiology, ETH, Zürich, Switzerland), described by Bollensen et al. (1990), were also used in our assays and were compared to our antibodies. In contrast to the antibodies P01–P07, they did not react with the extracellular domain of P0 in Western blot or ELISA (data not shown).

The main advantage of P01–P07 is their ability to recognize different partially defined epitopes of the extracellular domain of P0. P05–P07 react with mouse, rat, and human P0, indicating that they bind to epitopes which are highly conserved and might be of functional importance.

Antibodies to P0 are found during neuropathies associated with monoclonal gammopathies (Steck and Schluep, 1989; Hartung et al., 1992; Toyka and Hartung, 1992). Most of the antibodies described are directed against oligosaccharide residues on P0 and are thought to be responsible for the failure of myelin compaction observed in some of the neuropathies in these patients. Furthermore, there is evidence that antibodies directed to the protein backbone of the extracellular domain of P0 are present during experimental autoimmune (''allergic'') neuritis (EAN) and the Guillain-Barré syndrome (GBS; RAC Hughes, London, personal communication, and our own unpublished observations).

Currently we are using the antibodies P01–P07 in a sandwich ELISA to detect P0-ED-like immunoreactivity in serum and cerebrospinal fluid during GBS and its animal model EAN (Archelos et al., 1993). Interestingly, recombinant P0-ED is not neuritogenic in susceptible Lewis rats or rabbits, although the neuritogenic sequence alone contained in P0-ED (amino acid residues 56–71) is a strong neuritogen in both species (our own unpublished observations).

We believe that these monoclonal antibodies will be useful tools to investigate the role of P0 during myelination and in demyelinating diseases.

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