J1-160 and J1-180 Are Oligodendrocyte-secreted Nonpermissive Substrates for Cell Adhesion

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Abstract. The glia-derived J1 extracellular matrix glycoproteins have been referred to as J1-160/J1-180 (the developmentally late appearing lower molecular weight group) and J1-200/J1-220 (the developmentally early appearing higher molecular weight group immunochemically related to tenascin). Members of the two groups show distinct cross-reactivities. To characterize the structural and functional differences between these J1 glycoproteins, two monoclonal antibodies were generated which recognize only the members of the lower molecular weight group. The two antibodies detect immunochemical similarities among the members of the lower molecular weight group, but do not react with J1/tenascin. J1-160 and J1-180 are specifically expressed by differentiated oligodendrocytes in culture and by myelin of the central nervous system and have not been found in the peripheral nervous system nor in any other organ of the adult mice tested. Electron microscopic examination of rotary-shadowed J1-160 and J1-180 reveals, respectively, dimeric and trimeric (tribrachion) kink-armed rodlike structures, which are linked by disulfide bridges. J1-160/J1-180 are nonpermissive substrates for the attachment and spreading of early postnatal small cerebellar neurons, astrocytes, and fibroblasts. In a mixture with laminin, J1-160/J1-180 are nonpermissive substrates for neurons, but not for astrocytes or fibroblasts. The repulsive effect toward neurons can be neutralized by one of the monoclonal antibodies, but not by the other. These observations are discussed in the context of cell interactions during regeneration in the mammalian nervous system.

THE extracellular J1 glycoproteins were discovered in our laboratory as members of the family of adhesion molecules expressing the L2/HNK-1 carbohydrate. This was done by raising a polyclonal antibody against a 160-kD component of the "rest L2" glycoprotein fraction from adult mouse brain obtained by affinity chromatography on an L2 monoclonal antibody column after the sequential removal of the other members of the L2/HNK-1 family (N-CAM, L1, and myelin-associated glycoprotein [MAG])[1] by immunoaffinity chromatography (Kruse et al., 1984, 1985). This polyclonal antibody reacted with at least four different molecular species in mouse brain tissue which are differentially expressed during development. They were designated J1 glycoproteins based on the polyclonal antibody specificity (Kruse et al., 1985). These components are associated with and secreted by glial cells and fibroblasts and involved in neuron–glia, but not glia–glia, adhesion as measured by antibody perturbation experiments (Kruse et al., 1985; Seilheimer and Schachner, 1988). Polyclonal antibodies to J1 were found to specifically label the nodes of Ranvier in the adult rat optic nerve (ffrench-Constant et al., 1986). Since the J1 glycoproteins had been implicated in neuron–glia adhesion, they were suggested to be involved in the maintenance of the exquisite cytoarchitecture at the node of Ranvier. At the neuromuscular junction, J1 glycoproteins appear and accumulate within 2 d after denervation on basement membranes and fibrillar collagens in the vicinity of the original subsynaptic folds (Sanes et al., 1986). J1 glycoproteins are secreted by proliferating fibroblasts at the denervated site (Gatchalian et al., 1989) and disappear after successful reinnervation (Sanes et al., 1986), suggesting that their appearance at the denervated synapse is one of the signals involved in leading the reinnervating axon back to the original synapse (Sanes et al., 1986). Recently, J1 glycoproteins were found in the vibrissae-related barrel field boundaries of the somatosensory cortex of mice at the time of synapse formation between afferent nerve fibers and barrel field target cells (Steindler et al., 1989), suggesting that J1 may be involved in the shaping of afferent inputs. The use of polyclonal antibodies recognizing at least four distinct J1 glycoproteins did not allow us to determine unequivocally which of the molecular forms was responsible for these observations.

The J1 glycoproteins appear in many molecular forms with the predominant ones displaying apparent molecular masses (M_r) of 220, 200, 180, and 160 kD (Kruse et al., 1985). These molecular forms change in their expression during development of the central nervous system. The higher molec-

1. *Abbreviations used in this paper*: CMF-HBSS, Ca^{++} and Mg^{++}-free Hank's balanced salt solution; GFAP, glial fibrillary acidic protein; MAG, myelin-associated glycoprotein.

ular weight forms are expressed early and the lower molecular weight forms late during development (Kruse et al., 1985). The members of the high molecular weight group, J1-200 and J1-220, are immunochemically related to tenascin (Faissner et al., 1988), previously designated myotendinous antigen (Chiquet and Fambrough, 1984*a*,*b*), which is structurally related to hexabrachion (Erickson and Inglesias, 1984), human glioma-mesenchymal extracellular matrix antigen (Bourdon et al., 1983, 1985), and cytotactin (Grumet et al., 1985). These components will hereafter be designated J1/tenascin. Although the four molecular forms are distinct from each other, they display a certain immunochemical relatedness, since polyclonal antibodies adsorbed to one component can be retrieved from it and shown to react with other components (Faissner et al., 1988). Members of the J1/tenascin group, and the J1-180/J1-160 group are immunochemically more related to each other than to members of the other group (Faissner et al., 1988). However, knowledge on the functional properties of the individual components of J1 and their structural relationships to the others is lacking. It therefore seemed pertinent to produce monoclonal antibodies specific for a limited set, preferably for only one of the J1 glycoproteins.

Here we describe two monoclonal antibodies directed against components of the lower molecular weight group of J1. These components are specifically secreted by and associated with oligodendrocytes, but not astrocytes or fibroblasts, and are detectable in myelin of the central, but not peripheral, nervous system. When isolated in pure form and offered as substrate in vitro, they are nonpermissive for adhesion of neurons, astrocytes, and fibroblasts, and modify adhesion of neurons to its mixture with laminin. The repulsive effect of this substrate can be neutralized by one of the monoclonal antibodies but not by the other.

Materials and Methods

Antibodies

Monoclonal antibodies 596 and 597 recognizing J1-160 and J1-180 were obtained by the following immunization schedule of 4–6-wk-old BALB/c female mice. The L2 epitope-positive glycoprotein fraction from 0- to 2-d-old chicken brains obtained by immunoaffinity chromatography using an L2 (336) monoclonal antibody column (Kruse et al., 1984, 1985) was used as antigen. The glycoprotein fraction was preincubated overnight at 4°C with L2 monoclonal antibody 336 to block the very immunogenic L2/HNK-1 carbohydrate epitope. 10 μg of L2 epitope-positive glycoprotein fraction was then administered subcutaneously and intraperitoneally in complete (for the first injection) and incomplete Freund's adjuvant (for the three following injections) at intervals of 2 wk. Spleens of animals with high titers were selected and spleen cells fused with the mouse myeloma clone Ag8-653 (Kearney et al., 1979) according to the method of de St. Groth and Scheidegger (1980). Supernatants of antibody-producing hybridomas were screened by an ELISA assay on microtiter plates coated with the L2 epitope-positive glycoprotein fraction from 0- to 2-d-old chicken or adult mouse brains. Positive hybridoma cells were rescreened by the ELISA assay with "rest L2" from adult mouse brain (Kruse et al., 1984), containing predominantly the 160-kD form of J1 (Kruse et al., 1985; Faissner et al., 1988). The selected hybridomas were cloned twice by limiting dilution. The two monoclonal antibodies are of the IgG_1 subclass as tested by subclass-specific antibodies (Zymed Laboratories, CA). Antibodies were obtained as ascites either in BALB/c or nude mice or from serum-free hybridoma culture supernatants. Purification of immunoglobulin fractions of monoclonal antibodies from ascites or serum-free hybridoma supernatants was carried out on a protein A-Sepharose column (Pharmacia-LKB, Uppsala, Sweden) as described for mouse IgG_1 by the manufacturer.

The following antibodies were also used in our study: monoclonal L2 antibody 412 from rat, recognizing like the L2 antibody 336 a common carbohydrate epitope on several cell adhesion molecules (Kruse et al., 1984); monoclonal L1 antibody from rat, specific for subpopulations of postmitotic neurons in the central nervous system (Persohn and Schachner, 1987; Rathjen and Schachner, 1984); monoclonal antibodies 01 and 04 from mouse, specific for oligodendrocytes and immature glial cells (Kuhlmann-Krieg et al., 1988; Schachner et al., 1981; Sommer and Schachner, 1981; Trotter and Schachner, 1989); monoclonal antibody 513 from mouse to MAG (Poltorak et al., 1987); polyclonal antibodies from rabbit to MAG (a kind gift of F. Kirchhoff, University of Heidelberg, Heidelberg, FRG); polyclonal antibodies from rabbit to glial fibrillary acidic protein (GFAP) (a kind gift of Dr. L. Eng, Stanford University, Stanford, CA) recognizing the intermediate filament protein in differentiated astrocytes (Eng et al., 1971; Schachner et al., 1977); polyclonal antibodies from rabbit to J1/tenascin (a kind gift of Dr. A. Faissner, University of Heidelberg) recognizing the high molecular weight components of J1 which are immunochemically related to chicken tenascin (Chiquet-Ehrismann et al., 1986; Faissner et al., 1988). Polyclonal antibodies to the 180-kD band of J1-180 recognizing both J1-160 and J1-180, but not J1/tenascin, were produced in rabbits by three subcutaneous injections at 2-wk intervals with 50 μg of the 180-kD protein band obtained after electrophoretic transfer of J1-180 to nitrocellulose, first in complete then in incomplete Freund's adjuvant. Polyclonal rabbit antibodies to human plasma fibronectin and to the 200-kD chain of laminin from Englebreth-Holm-Swarm mouse sarcoma (both from Bethesda Research Laboratories, Gaithersburg, MD) were obtained by three subcutaneous immunizations at 2-wk intervals with 100 μg protein each, first in complete then in incomplete Freund's adjuvant.

Cell Cultures

Cell cultures of cerebella from 6- to 8-d-old NMRI mice and spinal cord from 15-d-old NMRI mouse embryos were prepared and maintained as described (Poltorak et al., 1987; Sadoul et al., 1989; Schnitzer and Schachner, 1982). Monolayer cultures of GFAP-negative and GFAP-positive astrocytes were obtained from 1- to 3-d-old mouse brains (Fischer, 1984; Fischer et al., 1982). Monolayer cultures of enriched oligodendrocytes and oligodendrocyte precursors were obtained from brains of 14-d-old mouse embryos (Trotter et al., 1989), Schwann cell cultures were prepared from sciatic nerves of 1-d-old mice (Seilheimer and Schachner, 1987), and dorsal root ganglion neurons were obtained from neonatal mice (Seilheimer and Schachner, 1988). Primary cultures of fibroblasts from embryonic mice were obtained according to Grumet et al. (1983) and maintained in Dulbecco's modified Eagle's medium containing 10% fetal calf serum.

Indirect Immunofluorescence

Fresh frozen sections of mouse brains, cerebella, and sciatic nerves were processed for indirect immunohistology as described (Goridis et al., 1978). Indirect immunofluorescence labeling of live- or fixative-treated monolayer cultures was performed according to methods previously described (Lagenaur et al., 1980; Schnitzer and Schachner, 1982; Schnitzer et al., 1981). By double immunofluorescence labeling with 04 (IgM) and 596 or 597 (IgG) monoclonal mouse antibodies, the monolayer cultures were first incubated with 04 antibody, followed by goat anti-mouse IgM-specific antibodies conjugated with TRITC, and then labeled with 596 or 597 antibody, followed by sheep anti-mouse IgG antibodies conjugated with FITC.

Immunoaffinity Purification of J1-160 and J1-180

Adult mouse brains (25 g) were homogenized in 400 ml of 20 mM Tris-HCl, 10 mM EDTA, 10 mM EGTA, 1 M urea, 1 μM aprotinin, 5 μM soybean trypsin inhibitor, and 1 mM phenylmethylsulfonyl fluoride, pH 7.9, at 4°C and the homogenate centrifuged for 30 min at 30,000 *g*. Supernatants were collected and precipitated in 40% ammonium sulfate for 2 h at 4°C. The precipitate was collected by centrifugation at 30,000 *g* for 30 min and dissolved in and dialyzed against PBS, pH 7.2, at 4°C. After clearing the dialyzed sample by centrifugation for 1 h at 100,000 *g* and 4°C, the supernatant was applied to a cascade of immunoaffinity chromatographic steps consisting of monoclonal antibody columns containing, in sequence, 513, 597, and 596 monoclonal antibodies covalently coupled to CNBr-activated Sepharose 4B (Pharmacia-LKB, Uppsala, Sweden) according to the manufacturer's instructions. Unbound material was washed from the columns with 20 bed volumes of 20 mM Tris-HCl, 1 mM EDTA, 1 mM EGTA, 0.5 M NaCl, 0.5% NP-40, pH 7.2, and the detergent removed by washing the columns extensively with PBS. J1-160 and J1-180 glycoproteins were eluted

from the 596 and 597 monoclonal antibody columns, respectively, with 0.1 M diethylamine, 0.1 M NaCl, 1 mM EDTA, and 1 mM EGTA, pH 11.2. The eluted fractions were quickly neutralized with 1 M Tris-HCl, pH 6.8, dialyzed against PBS and stored at −70°C in aliquots.

Analytical Procedures

Protein determinations were performed according to the method of Bradford (1976). Protein samples were analyzed by SDS-PAGE using 7% polyacrylamide slab gels by the method of Laemmli (1970) in the presence or absence of reducing agents. Proteins were visualized by the reducing silver staining method (Oakley et al., 1980). Western blot analysis was performed essentially as described (Towbin et al., 1979; Faissner et al., 1985) with the following modification: when tissue extracts were analyzed, the nitrocellulose filters were blocked after transfer for 1 h at room temperature in 50 mM Na_2HPO_4, 100 mM NaCl, 0.5% Tween 20, pH 8.4 (incubation buffer) containing 5% BSA followed by an overnight incubation at 4°C with first antibody in incubation buffer containing 1% BSA. Filters were then washed three times for 15 min each with incubation buffer and exposed to ^{125}I-protein A (Amersham-Buchler, Braunschweig, FRG; 1×10^6 cpm in 20 ml incubation buffer containing 1% BSA) for 1 h at room temperature. After subsequent washings, filters were developed by autoradiography using an enhancing screen and X-Omat AR film (Eastman Kodak Co., Rochester, NY).

Preparation of Tissue Extracts

Extracts from mouse brain and sciatic nerve were prepared by homogenization in 50 mM Na_2HPO_4, 100 mM NaCl, 0.2 mM $CaCl_2$, 0.2 mM $MgCl_2$, pH 7.4, containing 1% Triton X-100 and protease inhibitors (as described under immunoaffinity purification of J1-160 and J1-180) at a ratio of 1:5 (wt/vol) and incubation for 1 h on ice. The homogenates were then centrifuged for 30 min at 100,000 *g* and 4°C and supernatants frozen in aliquots at −70°C. Myelin fractions were obtained from adult mouse brain and sciatic nerve as described (Colman et al., 1982). For SDS-PAGE and Western blot analysis, fractions were washed twice in PBS and frozen in aliquots at −70°C.

Deglycosylation by Glycopeptidase F Treatment

Deglycosylation of immunoaffinity-purified J1-160 and J1-180 using glycopeptidase F (Boehringer Mannheim GmbH, Mannheim, FRG) was performed according to the manufacturer's instructions. Briefly, protein samples (50 μl at 100 μg/ml) containing 0.1% SDS and 1% β-mercaptoethanol were boiled for 5 min. Digestion buffer (40 μl of 20 mM Na_2HPO_4, 50 mM KH_2PO_4, 20 mM EDTA, 1% β-mercaptoethanol, pH 7.0) and enzyme solution (15 μl) were then added and incubated for 24 h at room temperature. Samples were then boiled in sample buffer for 5 min and subjected to SDS-PAGE and Western blot analysis as described.

Cell–Substratum Adhesion Assay

Cell culture substrates were prepared on nitrocellulose as described (Lagenaur and Lemmon, 1987). Protein substrates to be tested were applied in 2-μl droplets containing 20, 50, or 100 μg/ml protein in PBS. After 5-min adsorption to nitrocellulose, the droplets were removed by aspiration and the substrates blocked with Ca^{++} and Mg^{++}-free Hank's balanced salt solution (CMF-HBSS) containing 1% BSA for 60 min at room temperature. Alternatively, 10 μl of protein-containing solution was directly coated onto the plastic Petri dish, incubated for 60 min at 37°C, and unsaturated protein binding sites blocked as described for the nitrocellulose-mediated substrate adsorption. The Petri dishes were subsequently washed three times with CMF-HBSS or, in the antibody inhibition experiments, incubated for 30 min at 37°C with antibodies (50 μg/ml in CMF-HBSS) with subsequent washing as performed without addition of antibodies. The efficiency of coating of J1 glycoproteins individually or in mixture with laminin was measured by ELISA using monoclonal 596 and polyclonal antilaminin antibodies. In all cases, the expected protein fraction in the coated substrate was found. Single cell suspensions of freshly dissociated cerebellar cells from 6- to 8-d-old mice were prepared as described (Schnitzer and Schachner, 1982) and added to the substrate test plates at densities of 1×10^6 cells per ml in 2 ml of Eagle's basal medium containing 10% horse serum. For some experiments, small cerebellar neurons from 6- to 8-d-old mice were obtained by centrifugation through a Percoll (Pharmacia-LKB) gradient (Keilhauer et al., 1985). Astrocytes and embryonic fibroblasts were prepared as described in the Cell Cultures section. Astrocytes were also obtained by collecting the glial cell-enriched fraction of the Percoll gradient used to separate early postnatal cerebellar cells (Keilhauer et al., 1985). Astrocytes were used as probe cells in single cell suspensions after mild trypsin treatment of monolayer cultures (Kielhauer et al., 1985). Cell–substratum adhesion was evaluated after 1 h and process formation after 1.5 d in culture.

Rotary Shadowing and Electron Microscopy

The technique described by Tyler and Branton (1980) was used. J1 glycoproteins at a final concentration of 100 μg/ml were dialyzed against 20 mM Tris, 50 mM sodium acetate, pH 7.4, and adjusted to 50% (vol/vol) with glycerol. The solution (35 μl) was then sprayed onto a freshly cleaved chip of mica positioned 35 cm off the spray source. The mica chip was rotary shadowed in a Balzers S.p.A. (Milan, Italy) apparatus with tantalum/tungsten (80%/20%) or platinum/carbon (95%/5%) at an angle of 5° from an electron gun in a vacuum of 10^{-5} mbar. The thickness of metal deposits were controlled by a quartz crystal monitor. The cast was enforced by pure carbon, floated off onto distilled water, and mounted on 300-mesh copper grids. Microscopy was performed in a Philips 400 electron microscope at 60 kV under standardized magnification. Measurements of enlarged prints were made with an electronic graphics digitizer attached to a personal computer working with the Mosys program to compute distances and to construct histograms. For the determination of the absolute particle dimensions, the measured values had to be corrected for metal deposit thickness. These values were estimated by calculation (formula given by Balzers manual BB 800 048 BD) and by measurement of DNA as a reference molecule.

Results

Characterization of Two Monoclonal J1 Antibodies

In a preparation of L2/HNK-1 carbohydrate epitope-positive glycoproteins isolated from 0- to 2-d-old chicken brain (Fig. 1, lane *1*), the immunogen used for monoclonal antibody production, both 596 and 597 antibodies react with bands of apparent molecular masses of 160 and 180 kD (Fig. 1, lanes *6* and *11*). It is noteworthy that the two antibodies do not react with J1/tenascin (Fig. 1, lanes *6* and *11*), which is amply present in the L2/HNK-1 epitope-positive glycoprotein fractions from early postnatal brain (Faissner et al., 1988; Kruse et al., 1985). In a preparation of L2/HNK-1 carbohydrate, epitope-positive glycoproteins isolated from neonatal (Fig. 1, lane *2*) and adult (Fig. 1, lane *3*) mouse brain by immunoaffinity chromatography with an L2 antibody column, monoclonal antibody 596 reacts with bands of 160 and 180 kD (Fig. 1, lanes *7* and *8*) and monoclonal antibody 597 with a band of 180 kD (Fig. 1, lanes *12* and *13*). The two antibodies do not recognize other cell adhesion molecules of the L2/HNK-1 family, such as L1, N-CAM, or MAG (Fig. 1, lanes *5*, *10*, and *15*). They also do not react with glycoproteins of the extracellular matrix, such as J1/tenascin (220 and 200 kD) from adult mouse intestine or early postnatal mouse brain (Fig. 1, lanes *4*, *9*, and *14*), fibronectin, laminin, or secreted molecules of the endothelial cell line HR9 containing receptors for L1 and N-CAM (Werz and Schachner, 1988).

To isolate the respective antigens, 596 and 597 monoclonal antibody columns were prepared and used for purification of J1 glycoproteins from the supernatant of tissue homogenates of adult mouse brains in detergent-free buffer. Considerably less immunoreactive material could be purified from detergent extracts of the crude membrane fractions from adult mouse brain. The supernatant was passed, in sequence, over antibody columns containing the 513 monoclonal antibody to MAG and the antibodies 597 and 596. The corresponding

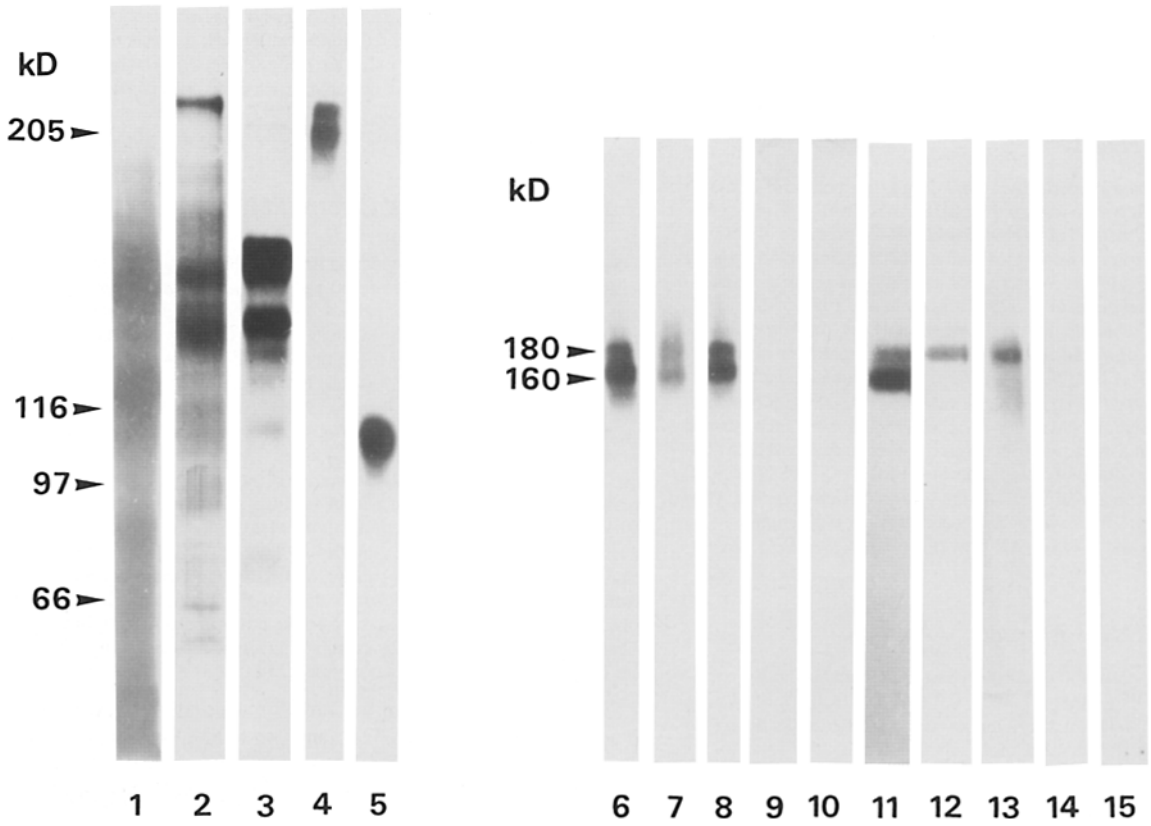


Figure 1. Characterization of specificities of 596 and 597 monoclonal antibodies by Western blot analysis using preparations of L2/HNK-1 carbohydrate epitope-positive glycoproteins immunoaffinity purified from 0- to 2-d-old chicken (lanes *1*, *6*, and *11*), neonatal (lanes *2*, *7*, and *12*) and adult (lanes *3*, *8*, and *13*) mouse brain, J1/tenascin from early postnatal mouse brain (lanes *4*, *9*, and *14*), and MAG from adult mouse brain (lanes *5*, *10*, and *15*). Samples were separated by SDS-PAGE under reducing conditions. Lanes *1–5* are glycoproteins visualized by the reducing silver method. For Western blot analysis, samples were transferred to nitrocellulose filters, incubated with 596 (lanes *6–10*) and 597 (lanes *11–15*) monoclonal antibodies, and immunoreactive bands detected by goat anti-mouse IgG antibodies conjugated with horseradish peroxidase.

antigens were recovered by elution under alkaline conditions. From the monoclonal antibody column 597, a glycoprotein fraction was obtained that contained, when analyzed by SDS-PAGE under reducing conditions, a dominant band at 180 kD and a weaker band at ~200 kD (Fig. 2, lane *1*). A high molecular weight smear, probably containing aggregated protein material, was occasionally seen at the top of the running gel (Fig. 2, lane *1*). Under nonreducing conditions this high molecular weight component was the only one seen (Fig. 2, lane *2*), indicating a high degree of disulfide linkage of protein chains. This antigen preparation was designated J1-180. The monoclonal antibody column 596 yielded a glycoprotein fraction containing one major component migrating at ~160 kD under reducing conditions (Fig. 2, lane *3*). Minor components, most probably degradation products, were sometimes seen at 125 and 145 kD (Fig. 2, lane *3*). Under nonreducing conditions a prominent band at ~320 kD and a weaker band at 160 kD were seen (Fig. 2, lane *4*). This preparation was designated J1-160.

To determine cross-reactivities between the two sets of antigens, the J1-180 and J1-160 glycoprotein preparations were tested by Western blot analysis with 596 and 597 monoclonal antibodies. In the J1-180 preparation, monoclonal antibody 596 recognized the predominant band at 180 kD and the less prominent component at ~200 kD (Fig. 2, lane *5*). Low levels of copurifying J1-160 were also recognized (Fig. 2, lane *5*). Monoclonal antibody 597 reacted in J1-180 preparation with the more prominent band at 180 kD and the weaker band at 200 kD (Fig. 2, lane *6*). These bands were strongly recognized by L2 monoclonal antibody 412 (Fig. 2, lane *7*) but not by polyclonal antibodies to J1/tenascin (Fig. 2, lane *8*). Monoclonal antibody 596 reacted with J1-160 (Fig. 2, lane *9*), whereas monoclonal antibody 597 did not react detectably with J1-160 (Fig. 2, lane *10*). L2 monoclonal antibody 412 also reacted well with J1-160 (Fig. 2, lane *11*), J1-160 was not recognized by the antibodies to J1/tenascin (Fig. 2, lane *12*). Under nonreducing conditions monoclonal antibody 596 reacted with two bands at 160 and 320 kD in the J1-160 preparation (Fig. 2, lane *13*). L2 monoclonal antibody 412 also reacted with these two bands under nonreducing conditions (Fig. 2, lane *14*).

To determine whether the antigenic determinants recognized by the two antibodies were protein or carbohydrate moieties, J1-160 and J1-180 were deglycosylated using glycopeptidase F and the resultant deglycosylated material was tested by Western blot analysis with monoclonal antibodies 596 and 597. In the deglycosylated J1-180 and J1-160 preparations, bands were shifted to lower molecular masses by ~20

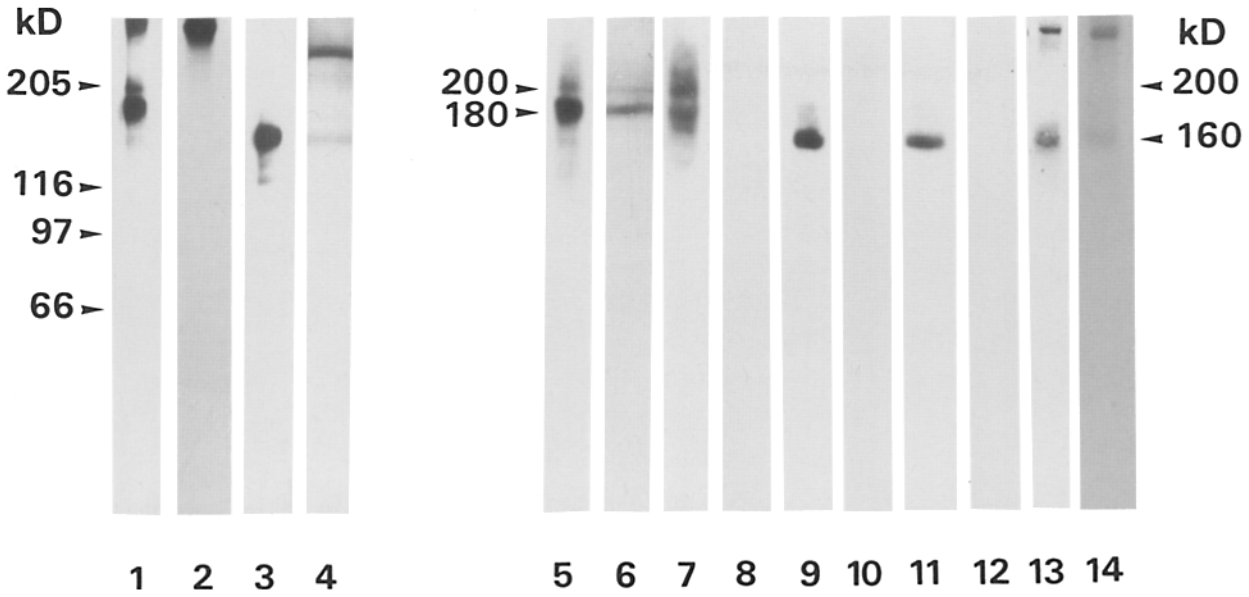


Figure 2. Western blot analysis of immunoaffinity-purified J1-160 and J1-180 from adult mouse brain using monoclonal antibody columns 596 and 597, respectively. J1-160 (lanes *3*, *4*, *9–14*) and J1-180 (lanes *1*, *2*, and *5–8*) glycoproteins were subjected to SDS-PAGE under reducing (lanes *1*, *3*, and *5–12*) and nonreducing (lanes *2*, *4*, *13*, and *14*) conditions. Lanes *1–4* represent J1-180 (lanes *1* and *2*) and J1-160 (lanes *3* and *4*), respectively, visualized by the reducing silver method. Glycoproteins were transferred to nitrocellulose filter (lanes *5–14*), and strips were cut and exposed to 596 (lanes *5*, *9*, and *13*), 597 (lanes *6* and *10*), and 412 (lanes *7*, *11*, and *14*) monoclonal antibodies, and to rabbit polyclonal antibodies to J1/tenascin (lanes *8* and *12*). Antibody binding was detected by goat anti-mouse IgG (for antibodies 596 and 597) and anti-rabbit IgG (for polyclonal antibodies to J1/tenascin) conjugated with horseradish peroxidase and rabbit anti-rat IgG conjugated with alkaline phosphatase (for antibody 412).

kD. The shifted bands were still recognized by the two antibodies, indicating that the two epitopes recognized by monoclonal antibodies 596 and 597 are not localized on *N*-glycosidically linked carbohydrate moieties (not shown). To verify whether other extracellular glycoproteins copurified with J1-160 and J1-180, the glycoprotein preparations were characterized by Western blot analysis for immunoreactivity with antibodies to fibronectin, laminin, or J1/tenascin. No immunoreactivity was found (Fig. 2, lanes *8* and *12*, for J1/tenascin).

Electron Microscopic Visualization of J1-160 and J1-180 by Rotary Shadowing

In J1-160 preparations a mixture of linear rodlike structures of equal thickness (~9 nm) was seen (Fig. 3, *A* and *B*). The length and frequency distributions in these antigen preparations revealed that 30% of the structures belong to a group with a 41.5-nm ± 10.5% average length and 60% of the structures belong to a group with a 97-nm ± 12.3% average length, with the remaining 10% of the structures not being allocated to one of these groups (Fig. 3 *F*). This frequency distribution was similar to the one seen under nonreducing conditions by SDS-PAGE (Fig. 2, lane *4*). Axial ratios of 1:11 and 1:25 were calculated for the short and long rod structures, respectively. Selected species out of 314 images examined are shown in Fig. 3, *A* and *B*. Short rods appeared with a predominant bending or hinge region at ~0.5 fractional length units of the rod and obvious polarity, with one end of the rod being slightly inflated (Fig. 3 *A*). Long rods appeared with a predominant bending or hinge region at ~0.25, 0.5, and 0.75 fractional length units of the rod (Fig. 3 *B*). Similar to the short rod structures, they also revealed polarity with one terminal region being stronger in contrast and thicker than the average diameter of the rods. Besides the described rods, some tribrachial stars were found, but with low incidence. The long rods disappeared after treatment with reducing agents resulting in short rod structures with the same appearance as described above (Fig. 3 *A*).

The J1-180 preparations consisted of tribrachial structures and very few linear rods, most likely degradation products of the tribrachion complex (Fig. 3, *C–E*). A gallery of selected structure images (Fig. 3, *C–E*) showed that the arms of the tribrachions were of approximately equal thickness (~8 nm), but unequal in length. The terminal regions of all three arms appeared stronger in contrast and slightly thicker than the internal regions. Length extensions of 56.6 ± 4.3, 49.9 ± 3.4, and 44 ± 4.5 nm for the long (Fig. 3 *E*, *l*), medium (Fig. 3 *E*, *m*), and short (Fig. 3 *E*, *s*) arms, respectively, were measured from 89 images. Length relations were calculated to 113:100:88. Internal bends or hinge regions are characteristic for all arms revealing their internal flexibility. The angles between the arms varied between 20° and 220°, but average openings were 120° for all angles. The sequences in which the arms emerged from the center of the structure were equal in their frequencies, suggesting that they are images of a unique structure adsorbed onto the mica in either front or back orientation. The tribrachion disintegrated into single rods in the presence of reducing agents, suggesting that the tribrachion is held together at its center by disulfide bridges. The formation of a higher molecular weight complex was confirmed by SDS-PAGE under nonreducing conditions (see Fig. 2, lane *2*). Hexabrachial struc-

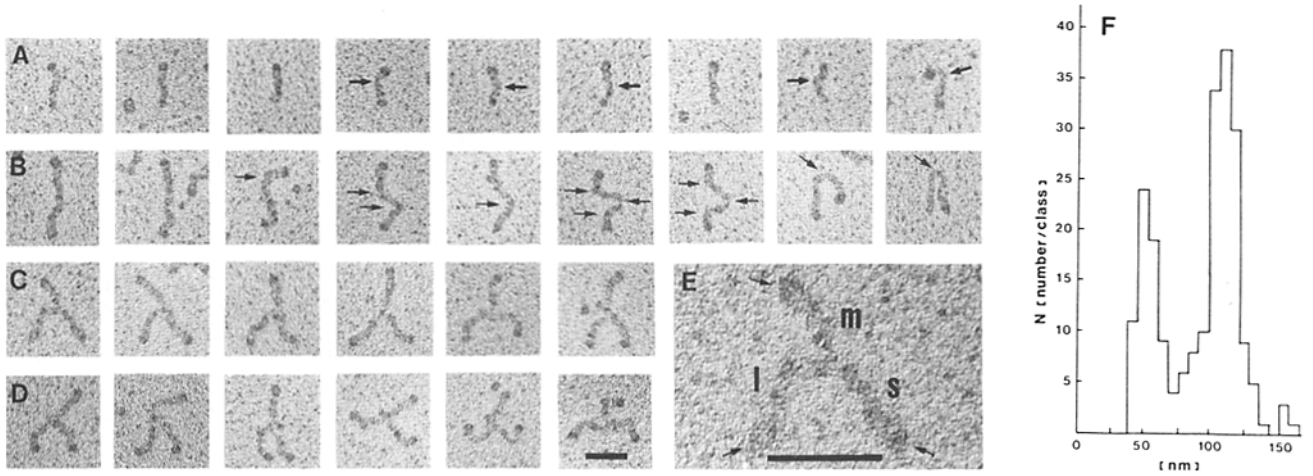


Figure 3. Electron micrographs of tantalum/tungsten rotary-shadowed Jl-160 with short rod structures of ~50 nm in length (*A*), long rod structures ~110 nm in length (*B*) (see *F* for length distribution of Jl-160 structures), and of Jl-180 (*C–E*). (*A*) The images are oriented in equal polarity with the thicker terminal region showing to the top. (*B*) The images are oriented with the thicker terminal region showing to the top. Frequent bending regions are indicated by arrows. (*C* and *D*) The different aspects of Jl-180 are shown. (*E*) Higher magnification view of the first image in *C*. The arms, long (*l*), medium (*m*), and short (*s*), are indicated. The terminal swellings are marked by arrows. (*F*) The length distribution of Jl-160 structures was evaluated from a total number of 314 measurements. The antigen preparation used was composed of 30% short and 60% long rod structures. Bars, 50 nm.

tures and/or complexes with more than three arms were never observed in Jl-180 preparations.

Developmental Appearance and Tissue Specificity of Jl-160 and Jl-180

The developmental appearance of Jl-160 and Jl-180 was studied in total brain and cerebellum of mice from embryonic day 12 to adult stages by Western blot analysis of tissue extracts using ^{125}I-protein A (Fig. 4). Antibody 596 was used to reveal both the Jl-160 and the Jl-180 components (Fig. 4, lanes *1–11*). In total brain tissue (Fig. 4, lanes *1–6*) Jl-180 was barely detectable at day 17 of embryonic development (Fig. 4, lane *1*) and increased in intensity until postnatal day 15 (Fig. 4, lane *5*), when it had reached adult levels (Fig. 4, lane *6*). Jl-160 only became detectable at postnatal day 5 (Fig. 4, lane *4*) to reach adult levels also at postnatal day 15 (Fig. 4, lanes *5* and *6*). In the cerebellum (Fig. 4, lanes *7–11*), Jl-180 was also the first component to appear (see postnatal day 0; Fig. 4, lane *7*). Between postnatal days 8 and 15 (Fig. 4,

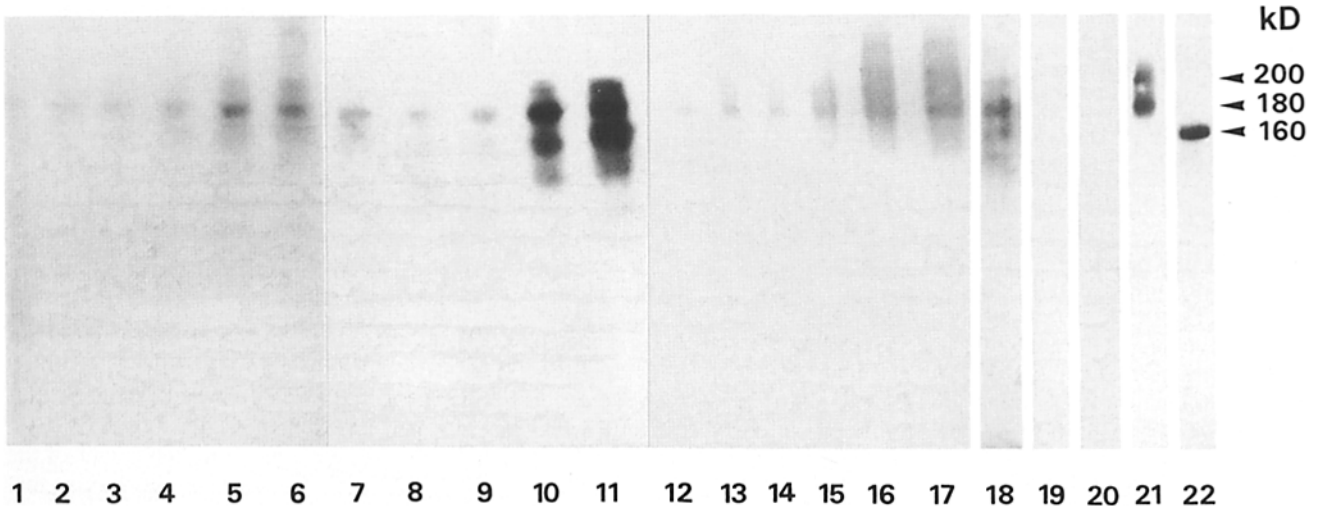


Figure 4. Western blot analysis of the developmental expression of Jl-160 and Jl-180 in brain (lanes *1–6* and *12–17*), cerebellum (lanes *7–11*), sciatic nerve (lane *19*), and myelin of central (lane *18*) and peripheral (lane *20*) nervous system of adult mice. Immunoaffinity-purified Jl-180 (lane *21*) and Jl-160 (lane *22*) serve as references. Equal amounts of protein (100 μg) from tissue homogenates of brains from 17-d (lanes *1* and *12*) and 19-d (lanes *2* and *13*) mouse embryos, neonatal (lanes *3* and *14*) mice, 7-d-old (lanes *4* and *15*), 15-d-old (lanes *5* and *16*), and >6-wk-old (lanes *6* and *17*) mice. Homogenates of cerebella from neonatal (lane *7*), 5- (lane *8*), 8- (lane *9*), 15- (lane *10*) day-old, and >6-wk-old (lane *11*) mice were separated by SDS-PAGE and subjected to Western blot analysis using 596 (lanes *1–11* and *18–22*) and 597 (lanes *12–17*) monoclonal antibodies. The Western blot was developed with ^{125}I-protein A.

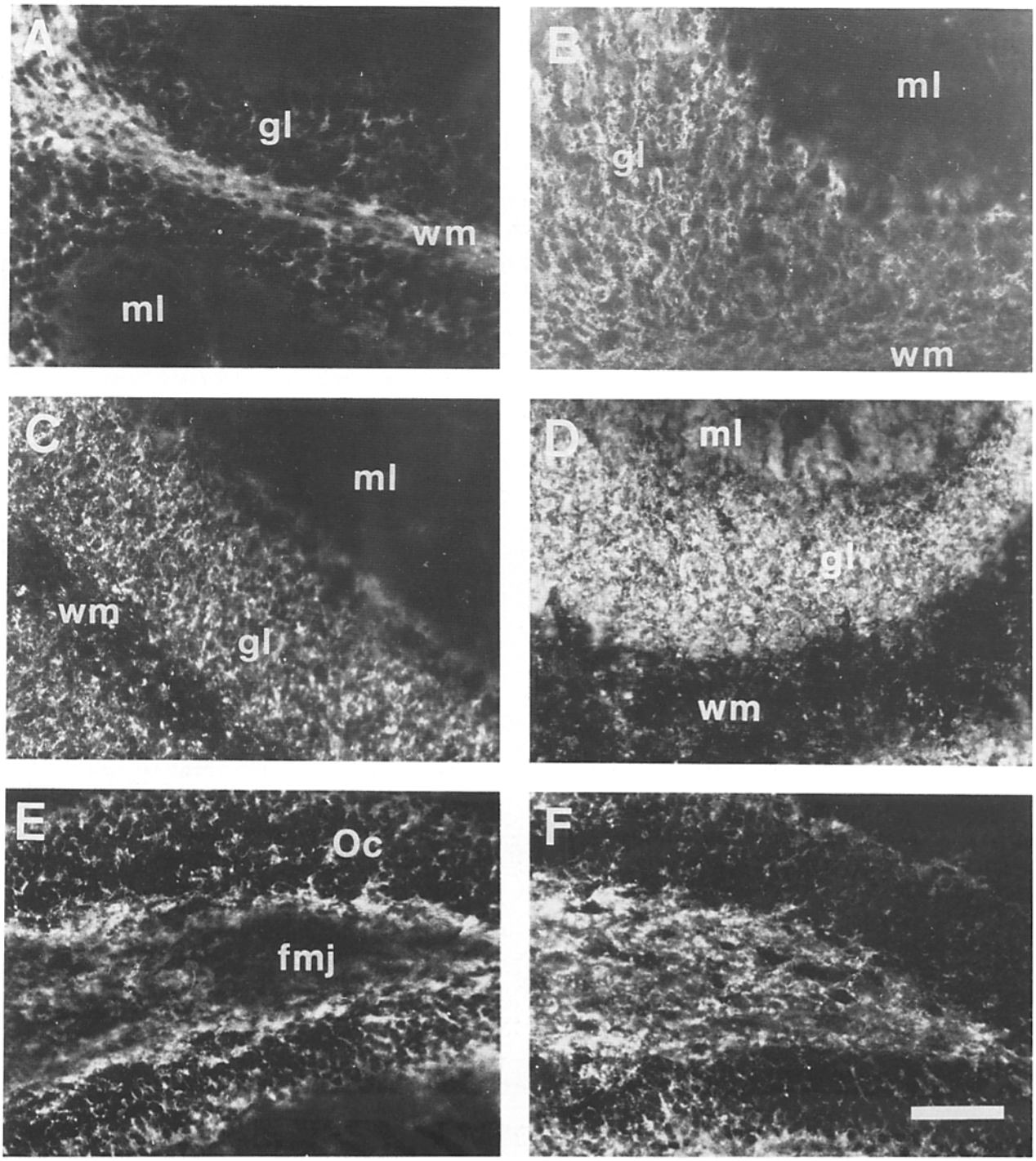


Figure 5. Immunohistological localization of J1-160 and J1-180 (*A* and *C–F*) and MAG (*B*) on fresh-frozen sections of 8-d-old (*A*) and adult (*B–D*) mouse cerebellum, and on sagittal sections of corpus callosum (*E* and *F*) in adult mouse brain by indirect immunofluorescence using 596 (*D* and *F*), 597 (*A*, *C*, and *E*), and 513 (*B*) monoclonal antibodies. Note the strong immunoreactivity for J1 in the prospective white matter of 8-d-old mouse (*A*) and its spotted distribution in the white matter of adult (*C* and *D*) animals. In the adult cerebellum, J1-180 is strongly expressed in the granular and Purkinje cell layers, while the molecular and external granular layers are J1-180 negative (*A* and *C*). J1-160 shows the same expression pattern and appears to be additionally localized in the molecular layer (*D*). In corpus callosum of adult mouse J1-160/J1-180 is expressed on myelinated callosal axons and the adjacent occipital cortex (*E* for 597 and *F* for 596 antibody). Note that MAG is more uniformly distributed in the white matter of adult cerebellum (*B*) than J1-160/J1-180 (*C* and *D*). *fmj*, Forceps major corpus callosum; *gl*, granular layer; *ml*, molecular layer; *Oc*, occipital cortex; *wm*, white matter. Bar, 100 μm.

lanes *9* and *10*) a large increase in the amount of J1-180 and a comparable presence of J1-160 were seen (Fig. 4, lane *10*) almost reaching adult levels (Fig. 4, lane *11*). Some smaller immunoreactive bands were sometimes seen in the range of 125 and 145 kD (Fig. 4, lanes *5*, *6*, *10*, and *11*) reminiscent of those in the J1-160 preparations and possibly representing degradation products. When monoclonal antibody 597 was used to examine brain tissue extracts by Western blot analysis

of the same ages used for monoclonal 596 antibody (Fig. 4, lanes *12–17*), confirming results were obtained in that J1-180 was barely detectable at embryonic day 17 (Fig. 4, lane *12*) and increased in intensity until postnatal day 15 (Fig. 4, lane *16*) when it had reached adult levels (Fig. 4, lane *17*). The higher molecular weight components recognized by 597 antibody in tissue extracts from postnatal day 15 (Fig. 4, lane *16*) and adult (Fig. 4, lane *17*) animals likely represent polymers of J1-180, resulting from partial reduction of disulfide bonds. The same results as those seen with 596 antibody were obtained by polyclonal antibodies to J1-180. J1-160 and J1-180 were present in purified myelin from adult mouse brain (Fig. 4, lane *8*), but not in tissue homogenate (Fig. 4, lane *19*) or myelin fraction (Fig. 4, lane *20*) from adult mouse sciatic nerve.

Using 596 and 597 monoclonal antibodies and polyclonal antibodies to J1-180 which recognize both J1-160 and J1-180, the J1-160 and J1-180 glycoproteins have not been detected by the sensitive ELISA and Western blot tests in extracts of several nonneural tissues of adult mice, including skin, kidney, intestine, liver, lung, spleen, skeletal muscle, heart, and blood. The J1-160 and J1-180 glycoproteins were detectable in optic nerve and spinal cord, however, indicating that J1-160 and J1-180 are uniquely associated with the central nervous system.

Immunohistological Localization of J1-160 and J1-180 during Development

Sagittal sections of fresh frozen mouse cerebellum at different developmental stages and adult mouse forebrain were investigated by indirect immunofluorescence using antibodies 597 and 596 (Fig. 5). The expression of J1-160/J1-180 was studied at 0, 5, 7, 8, 10, 15, and 21 d after birth and in the adult. At birth, no detectable immunoreactivity was observed with both antibodies. Weak immunoreactivity was seen 5 d after birth in the prospective white matter (not shown). At

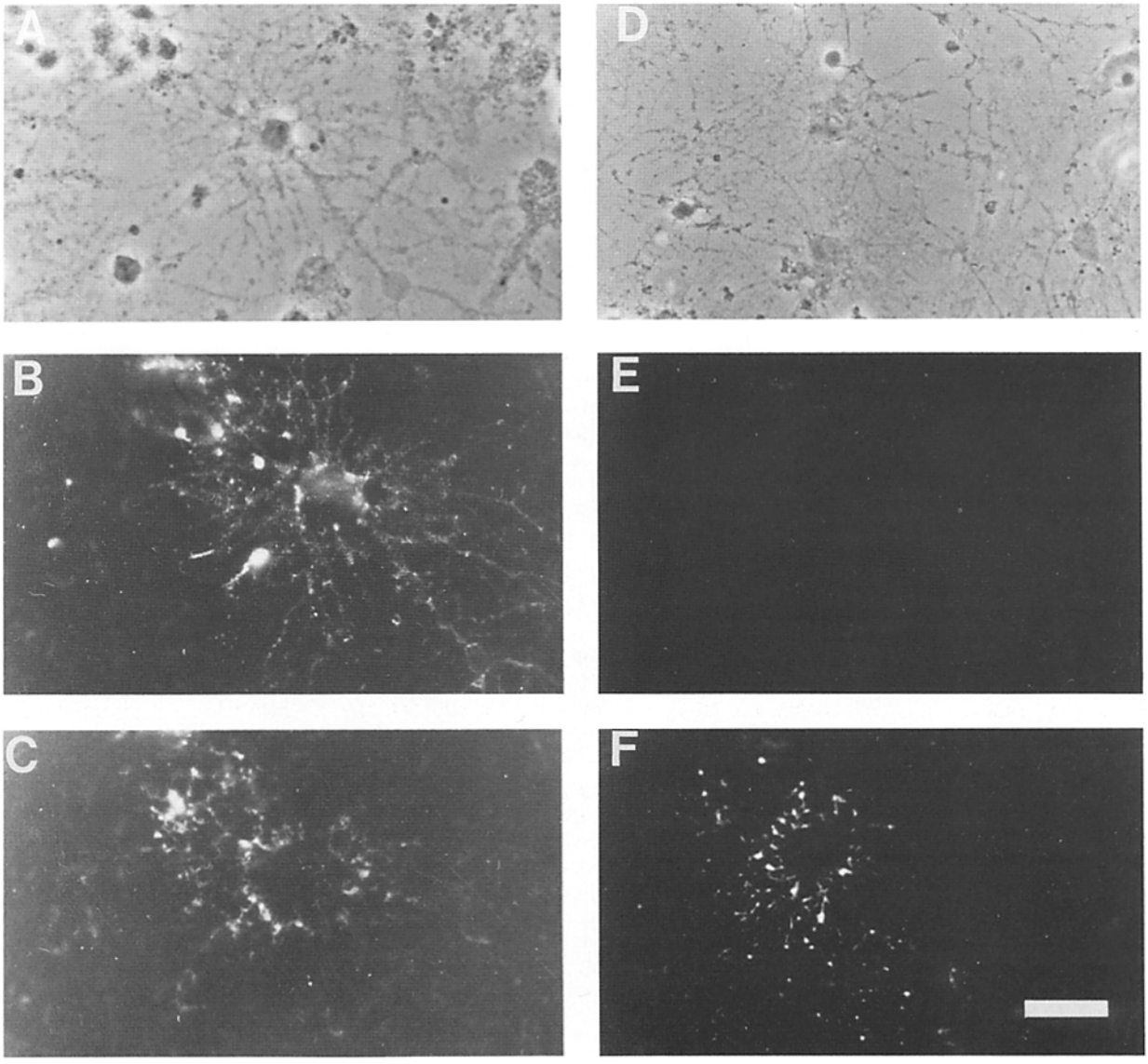


Figure 6. Double immunofluorescence labeling of monolayer cultures from 6-d-old mouse cerebellum maintained for 7 d in vitro using 596 (*C* and *F*) and 04 (*B*) monoclonal antibodies and rabbit polyclonal antibodies to MAG (*E*). *A* and *D* are the corresponding phase contrast micrographs to fluorescence images *B* and *C*, and *E* and *F*, respectively. Note the patchy distribution of J1-160/J1-180 associated with differentiated oligodendrocytes on the cell body and processes (*C* and *F*) and the absence of MAG immunoreactivity on a J1-160/J1-180-positive oligodendrocyte (*E*). Bar, 30 μm.

postnatal day 8, the antibodies reacted strongly with the developing white matter in a rather uniform labeling pattern, leaving cell bodies spared (see Fig. 5 *A* for antibody 597). The internal granular layer was only weakly stained at this age, while the molecular and external granular layers were not immunoreactive (Fig. 5 *A*). At day 15, the pattern and intensity of immunofluorescence staining was similar to that of the adult (Fig. 5, *C* and *D*). As at earlier developmental stages, J1-180 was not found in the molecular layer and was only weakly or not at all detectable in the Purkinje cell layer (Fig. 5 *C*). J1-160 was additionally detectable in the molecular layer (Fig. 5 *D*), and in the internal granular layer its labeling pattern was similar to that of MAG (Fig. 5 *B*). Interestingly, J1 immunoreactivity was not uniformly detectable in white matter tracts, but in randomly distributed fluorescent dots (Fig. 5, *C* and *D*), while MAG showed a more uniform labeling pattern in adjacent sections (Fig. 5 *B*; Poltorak et al., 1987). Also in the cerebral hemispheres, J1-160/J1-180 was found predominantly in white matter and with a time course of increasing immunofluorescence staining intensities with development. The staining patterns of antibodies 596 and 597 were compared in sagittal sections of adult forebrain (Fig. 5, *E* and *F*). Both J1-160 and J1-180 were detectable in the myelinated tracts of the corpus callosum and in the occipital cortex, but not in unmyelinated cortical layers.

Sections of fresh frozen or paraformaldehyde-treated sciatic nerve or intestine from adult mice did not show detectable staining with 596 and 597 antibodies (not shown), in agreement with the immunochemical analysis by ELISA and Western blot.

Immunocytological Localization of J1-160 and J1-180 in Cell Cultures

Cell type-specific expression of the antigens was studied in monolayer cultures of early postnatal mouse cerebellum, spinal cord, and sciatic nerve. In the cerebellar cultures, the morphologically differentiated oligodendrocytes (hairy eyeball cells) with an extensive network of processes were labeled by antibodies 596 and 597 (Fig. 6, *A–C*). The identity of the immunopositive cells as oligodendrocytes could be verified by double immunolabeling with the monoclonal antibody 04 reacting with glial precursor cells and oligodendrocytes (Fig. 6, *A–C*). The appearance of J1-160- and J1-180-positive structures was always spotty, appeared extracellularly localized, and was less continuous than the labeling with 04 antibody (Fig. 6 *B*). Oligodendrocyte precursors and more immature oligodendrocytes with small cell bodies and a bi- or tripolar organization of their processes were never found to label with antibodies 596 and 597. No overlap of MAG-positive and J1-160/J1-180-positive hairy eyeball oligodendrocytes could be observed by double immunofluorescence in these cultures (Fig. 6, *D–F*). Thus, not all oligodendrocytes expressed J1. Other cell types present in these cultures, such as fibronectin-positive fibroblasts, L1-positive neurons, and GFAP-positive astrocytes, were never observed to stain detectably with 596 and 597 antibodies.

In monolayer cultures of spinal cord from 15-d-old mouse embryos, no labeling for J1 was detectable after 1 wk in culture, while an intensive immunofluorescence staining on oligodendrocytes could be seen after 2 wk in culture (not shown).

In Schwann cell cultures of sciatic nerve from early postnatal mice, no labeling was seen with the two antibodies (not shown). When Schwann cells were cocultured with dorsal root ganglion neurons for up to 2 wk, Schwann cells remained J1 antigen negative, while MAG could be detected after 10 d in vitro, indicating that Schwann cells can be induced to express differentiation markers in vitro.

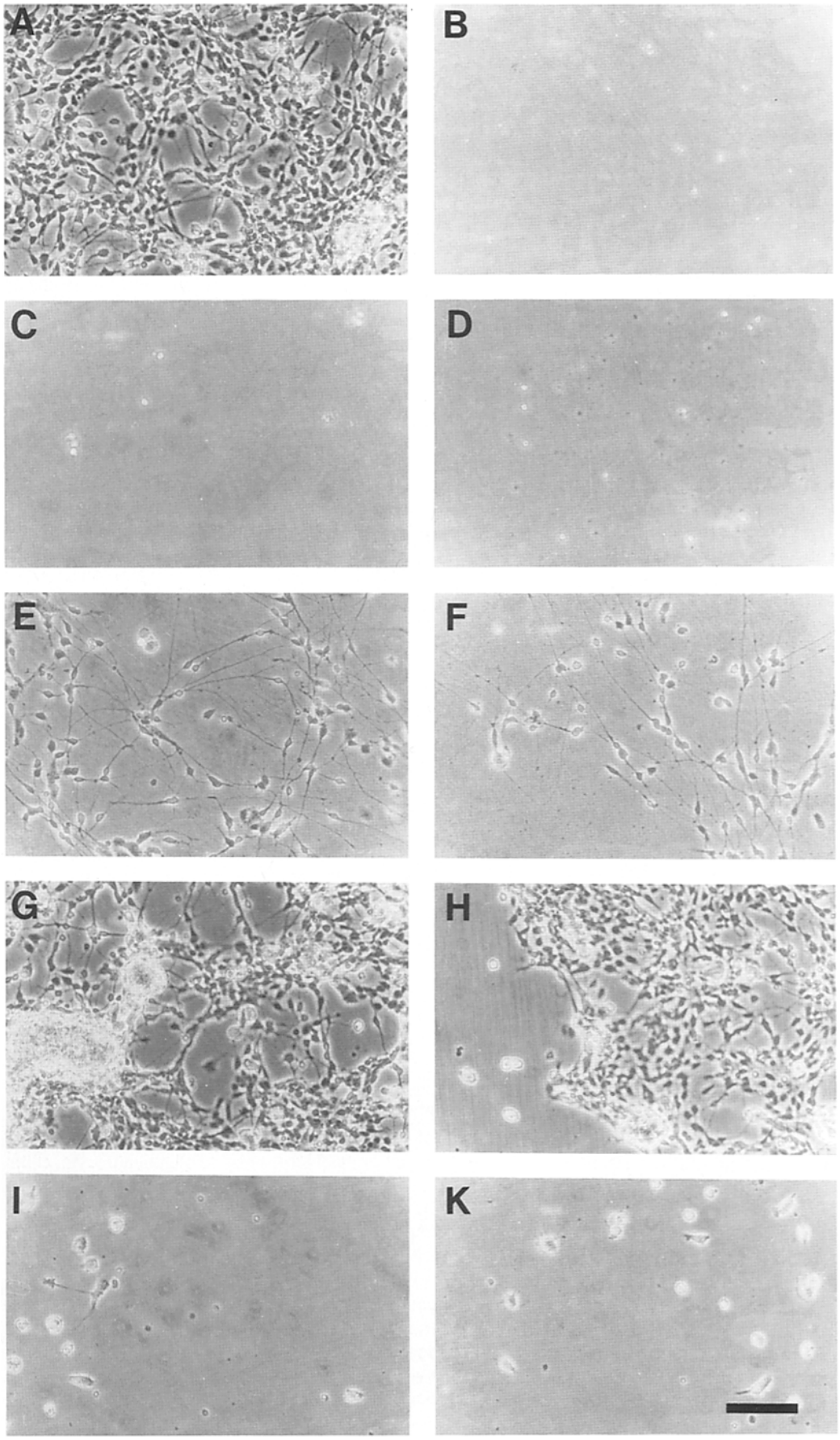
Cell Adhesion and Process Formation on J1-160 and J1-180 Substrates

To investigate whether the extracellular matrix constituents J1-160 and J1-180 are good substrates for cell adhesion and process formation, purified antigens (50 μg/ml) were coated as substrate on a layer of nitrocellulose. Alternatively, the purified glycoproteins were directly coated onto plastic surfaces. Cell adhesion, cell spreading, and extension of processes from single cell suspensions of early postnatal cerebellum was measured after 24–30 h in vitro. As positive and negative controls, laminin (50 μg/ml) and BSA (50 μg/ml) respectively, were coated in the same fashion as the J1 substrates.

Freshly dissociated single cells from 7-d-old mouse cerebella attached well to laminin and elaborated a dense network of processes within ~1 d in culture (Fig. 7 *A*). These cells did not attach to BSA (Fig. 7 *B*). J1-160 (Fig. 7 *C*) and J1-180 (Fig. 7 *D*) were equally bad substrates. As a next step, cell spreading and process outgrowth were measured on a mixture of laminin and J1-160 (Fig. 7, *E* and *G*) or J1-180 (Fig. 7, *F* and *H*) in a ratio of 1:1 (wt/wt) at the same final protein concentration as the two substrates individually (50 μg/ml; Fig. 7, *A*, *C*, and *D*). Fewer cells attached and extended processes on this substrate than on laminin, but significantly more than on J1-160 or J1-180 alone. To exclude the possibility that a mixture of laminin with any protein could alter cell behavior toward laminin, BSA and MAG were tested in a mixture with laminin and found to have no effect on the rapid adhesion of cerebellar cells to the substrate. To investigate whether the adhesion-nonpermissive properties of the J1 molecules could be neutralized on the laminin–J1 substrates, all substrates were preincubated with 596 antibody before addition of cells. Under these conditions cerebellar cells attached as well to the mixture substrate as to laminin alone (Fig. 7, *G* and *H*), but did not attach to the J1 substrate. Antibody 597 did not show any neutralizing effect nor did IgG fractions obtained from nonimmune rats or mice (not shown). To check for the possibility that adsorption of the two antibodies onto the substrate could alone have caused cell attachment, antibody substrates were monitored for adhesion of cells (Fig. 7, *I* and *K*). Only very occasionally an adherent cell probably a J1-positive oligodendrocyte, could be seen under these conditions.

Basically similar results were obtained when purified small cerebellar neurons from 7-d-old mouse cerebellum, the major cell population at this postnatal stage, were plated as single cells onto these substrates. Again, laminin was the expected good substrate, while BSA, J1-160, and J1-180 were bad substrates. Antibody 596 neutralized the adhesion-nonpermissive effects of the J1-160 and J1-180 substrates in a mixture with laminin.

The effects of J1-160 and J1-180 on attachment and spreading of astrocytes purified from 8-d-old mouse cerebellum were different from those seen with neurons (Fig. 8). While the cells adhered and spread rapidly on laminin (Fig. 8 *A*), they attached only loosely on J1-160 and J1-180 and remained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round (Fig. 8, *C* and *D*). In contrast, the mixture of J1-160 and J1-180 with laminin produced good adhesion and spreading of astrocytes (Fig. 8, *E* and *F*). The same effects were seen with astrocytes isolated from cerebral hemispheres of neonatal mice. Preincubation of substrates with antibody 596 or 597 did not alter astrocyte adhesion and spreading.

The behavior of small cerebellar neurons and cerebellar astrocytes did not differ, when different developmental stages of cells or different protein concentrations of J1 substrates (between 10 and 100 μg/ml) were used. Also, the nonpermissive effect on neuronal adhesion did not depend on the ratios of laminin and J1 mixture over a broad range tested. However, smaller amounts of J1 (10 μg/ml) in the mixture with laminin (90 μg/ml) did not produce a nonpermissive effect.

When a nonneural cell type (i.e., embryonic mouse fibroblasts) was used in control experiments, J1-160 and J1-180 did not influence fibroblast adhesion and spreading in a mixture with laminin.

Discussion

By generating the two monoclonal antibodies 596 and 597 we have been able to dissect the molecular heterogeneity of the J1 glycoprotein complex into immunochemically distinct subgroups. The two monoclonal antibodies against components of the lower molecular weight group (J1-160 and J1-180) do not react with components of the higher molecular weight group (J1/tenascin, consisting of 200- and 220-kD components), thus underscoring the molecular differences between these two groups. This immunochemical dissection of the J1 complex into two distinct subgroups is supported by immunocytological evidence showing that J1-160 and J1-180 are restricted in expression to oligodendrocytes, whereas J1/tenascin is expressed by astrocytes, fibroblasts, and Schwann cells. Polyclonal J1 antibodies recognizing all four components of the two groups had previously been reported to label oligodendrocytes as well as astrocytes and fibroblasts (Kruse et al., 1985).

An antigenic relatedness between the components of the lower molecular weight group is revealed by the two monoclonal antibodies. Of the two distinct protein epitopes recognized by the monoclonal antibodies 596 and 597, one is present on J1-160 and J1-180 (596 antibody) and the other only on J1-180 (597 antibody). J1-160 and J1-180 can be separated from each other, although they are immunochemically related, by taking advantage of sequential immunoaffinity chromatographic purification. After removing first the J1-180 component from extracts of adult brain tissue, the remaining J1-160 can be isolated. It should be noted that J1-180 comprises mainly two molecular components of 180 and 200 kD. The higher molecular weight component of J1-180 has essentially the same molecular mass as the J1/tenascin component, but is antigenically distinct from it, since monoclonal antibodies to J1-180 (596 and 597) do not react with the 200-kD component of J1/tenascin and polyclonal antibodies to J1/tenascin do not react with the higher molecular weight band in preparations of J1-180. It should be mentioned that J1/tenascin is not or is only weakly present in adult mouse brain (Faissner et al., 1988; Kruse et al., 1985) which is the source of J1-180. Furthermore, 596 and 597 monoclonal antibodies do not recognize any of the other neural adhesion molecules belonging to the L2/HNK-1 family (Kruse et al., 1984).

The two components, J1-160 and J1-180, exist in different molecular forms depending on the presence of disulfide bridges, since J1-160 dimerizes and J1-180 trimerizes under nonreducing conditions. The multimeric forms of oligodendrocyte-specific, L2/HNK-1-positive glycoproteins in the human central nervous system, most likely related to J1-160 and J1-180, have previously been reported to be linked by disulfide bonds (Gulcher et al., 1986). Electron microscopic examination of rotary-shadowed J1-160 and J1-180 visualize the different structures of the two glycoprotein components. J1-160 consists of short and long kink-armed rods with globular terminal domains. They most likely represent monomers and dimers since both structures have the same appearance and immunochemical characteristics. The monomers appear to associate at their nonglobular ends via disulfide bridges, since reducing agents disintegrate dimers to monomers. J1-180 is a three-armed structure or tribrachion with each arm slightly different in length from the others. The three arms can be dissociated by reducing agents, suggesting that they are also held together in the tribrachion center by disulfide bridges. It remains to be seen which molecular forms of J1-180 revealed by SDS-PAGE correspond to the different arm lengths observed in the electron micrographs of the protein. In SDS-PAGE the two components of J1-180 (180 and 200 kD) are not present in equal amounts and per se cannot correspond to three different arm lengths. Therefore, it is more likely that differences in length reflect differences in the three-dimensional structure of the arms.

Both J1-160 and J1-180 are specifically expressed by oligodendrocytes, but not by any other cell type studied so far, including other glial cells in the central and peripheral nervous system. The J1-160- and J1-180-positive oligodendrocytes belong to a subgroup of more differentiated oligodendrocytes that do not express MAG under the culture conditions used. The two J1 components are not detectable on undifferentiated oligodendrocytes or oligodendrocyte precursor cells and appear relatively late during brain development, with J1-180 being expressed earlier than J1-160, but both are present before the onset of myelination. This pattern of expression is interesting from the point of view that polyclonal J1 antibodies and monoclonal L2/HNK-1 antibodies react uniquely with the node of Ranvier in histological sections of adult rat optic nerve (ffrench-Constant et al., 1986). Although the polyclonal J1 antibodies used in this study were not specific for J1-160 or J1-180, immunoreactive glycopro-

Figure 7. Demonstration of adhesion-nonpermissive properties of J1-160 and J1-180 for cerebellar cells and neutralization by monoclonal antibody 596. Test substrates (*A*, laminin; *B*, BSA; *C*, J1-160; *D*, J1-180; *E* and G, mixture of laminin with J1-160 [1:1 on a protein basis]; *F* and *H*, mixture of laminin with J1-180 [1:1 on a protein basis]; *I*, IgG fraction of monoclonal antibody 596; and *K*, IgG fraction of monoclonal antibody 597) were adsorbed on a nitrocellulose layer and single cell suspensions of cerebella from 7-d-old mice were added to the Petri dishes containing spots of all test substrates. Cultures were examined 24–30 h after plating of single cells. *G* and *H*, substrate spots were preincubated with 596 antibody before the addition of single cells. Bar, 100 μm.

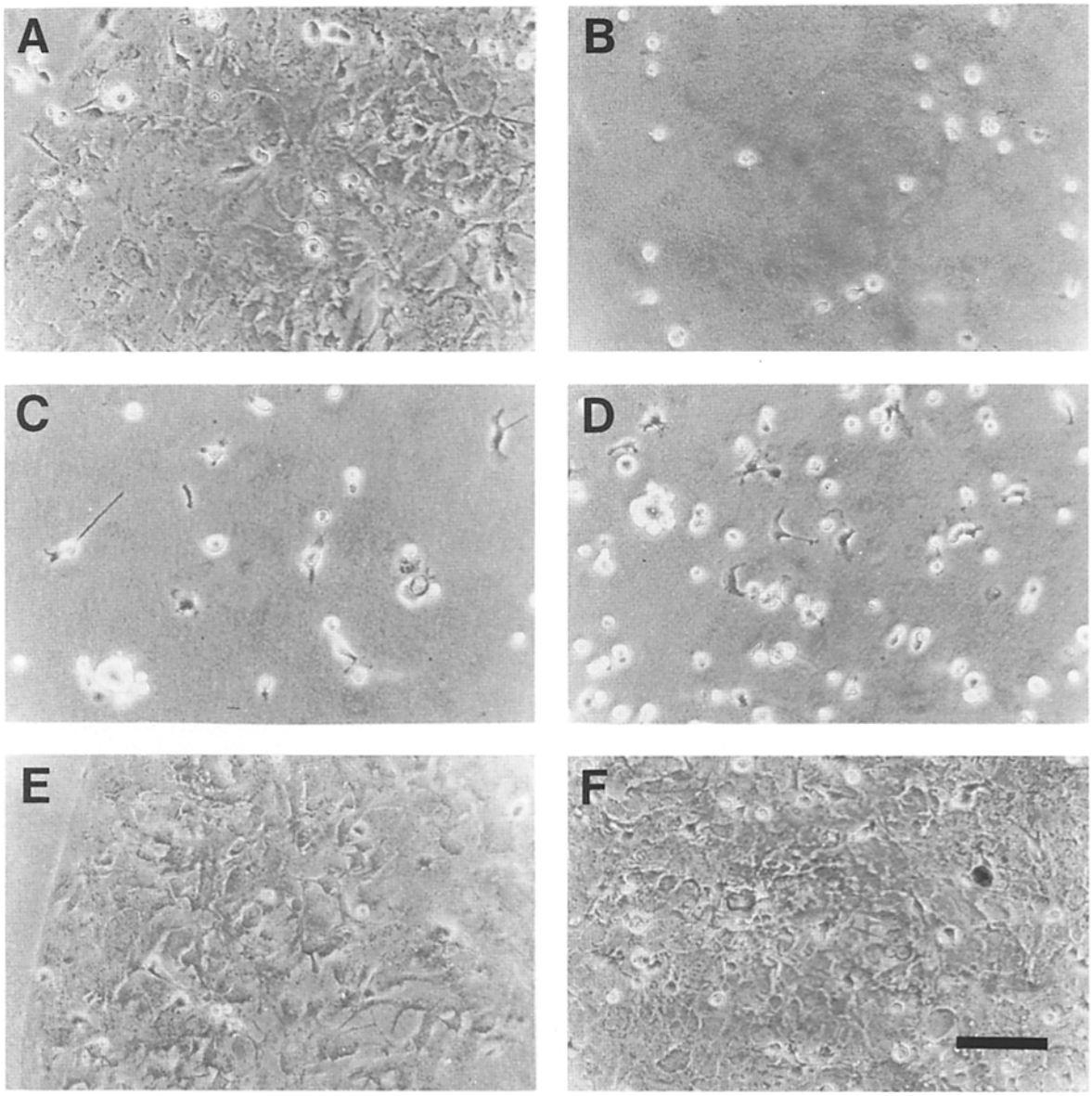

Figure 8. Demonstration of adhesion-nonpermissive properties of J1-160 and J1-180 for astrocytes. Test substrates (*A*, laminin; *B*, BSA; *C*, J1-160; *D*, J1-180; *E*, mixture of laminin with J1-160 [1:1 on a protein basis]; and *F*, mixture of laminin with J1-180 [1:1 on a protein basis]) were adsorbed on a nitrocellulose layer and single cell suspensions of astrocytes from 8-d-old mouse cerebella were added to the Petri dishes containing the substrate spots. Cultures were examined 24 h after plating of single cells. Note the border of the substrate spot at the left upper margin in *A* and left margin in *E*. Bar, 100 μm.

teins in the adult rat optic nerve predominantly belonged to the lower molecular weight components of the J1 complex. Therefore, the J1 components at the node of Ranvier are most likely J1-160 and/or J1-180. It is interesting in this context that monoclonal antibodies 596 and 597 label white matter tracts of cerebellum and cerebral hemispheres in a dot-like manner. Unfortunately, all attempts to use the two antibodies for immunoelectron microscopic procedures have so far failed (Bartsch, U., unpublished observations). Furthermore, it remains to be seen whether oligodendrocytes or type II astrocytes, which derive together with oligodendrocytes from a common precursor cell (ffrench-Constant and Raff, 1986) and interdigitate with their processes into the node of Ranvier secrete J1-160 or J1-180 at this specialized region (Bartsch, U., P. Pesheva, and M. Schachner, unpublished observations). Alternatively, the putative receptors for the two components may specifically localize there.

A surprising finding of our study are the adhesion-nonpermissive substrate properties of purified J1-160 and J1-180 for cells that are physiological partners of oligodendrocytes, such as neurons and astrocytes, but also for nonphysiological partner cells, such as fibroblasts. The repulsive properties for neurons can be neutralized by one of the monoclonal antibodies when the J1 glycoproteins are mixed with laminin, suggesting that other adhesive mechanisms remain intact on the assay cells. Repulsive effects towards mammary epithelial cells and fibroblasts have been described for tenascin (Chiquet-Ehrismann et al., 1988). However, previous evi-

dence from our laboratory suggests that J1/tenascin is adhesive, since Fab fragments of polyclonal J1 antibodies interfered with attachment of neurons to astrocytes (Kruse et al., 1985). Since in the previous study oligodendrocytes were not tested for J1-dependent adhesion to neurons, we do not know whether the astrocytic forms of J1 have functional properties different from those of oligodendrocytes or whether other factors are involved. Nonpermissive properties of cell surface glycoproteins characteristic of differentiated oligodendrocytes have recently been described (Caroni and Schwab, 1988*a*,*b*; Schwab and Caroni, 1988). The two glycoproteins characterized by Schwab and colleagues show features reminiscent of J1-160 and J1-180: (*a*) they are expressed by morphologically differentiated oligodendrocytes in culture, (*b*) their nonpermissive properties can be neutralized by monoclonal antibodies, (*c*) the nonpermissivity pertains to physiological and nonphysiological partner cells, and (*d*) the molecules are expressed in the central but not in the peripheral nervous system. It is likely, however, that J1-160 and J1-180 are distinct from these glycoproteins, since they are not membrane bound, but soluble under physiological conditions. Furthermore, preliminary immunochemical comparisons indicate distinct differences between the two groups of molecules (Pesheva, P., M. E. Schwab, and M. Schachner, unpublished observations). These combined observations suggest the existence of several molecules expressed by differentiated oligodendrocytes and central nervous system myelin that are involved in adhesion-nonpermissive effects. The observations also raise questions as to the differences, but also similarities, of such molecules and their functional significance during development and under conditions where regeneration is called for.

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