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Monoclonal antibody cross-reactions between *Drosophila* and human brain

(neural antigens/species homology/molecular anatomy/immunohistology)

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ABSTRACT **A panel of 146 monoclonal antibodies (MAbs), obtained with *Drosophila melanogaster* tissue as primary immunogen, was tested for cross-reactivity with the human central nervous system. Sites examined included spinal cord, cerebellum, hippocampus, and optic nerve. Nonnervous tissues tested were liver and lymph node. Approximately half of the antibodies reacted with one or more sites in the human central nervous system, identifying regional, cell class, and subcellular antigens. Some recognized neuronal, glial, or axonal subsets. Immunoblot analysis revealed that some antibodies reacted with similar antigen patterns in both species.**

Although much of the neuronal network of the human central nervous system has been mapped by classical histologic and electrophysiologic methods, our knowledge of molecular differences between functional cellular subsets is limited. A remarkable tool, the monoclonal antibody (MAb), provides a new dimension to neuroanatomy. This technique has begun to reveal, in the central nervous system of various organisms, hitherto unsuspected subsystems identifiable by their molecular specificities (1–4). Although there has been some application of MAbs to the human central nervous system, only a few surveys of human neural antigens have been reported (5–8), partly due to the difficulty of obtaining appropriate tissues and the complexity of the structure.

Using a panel of 146 MAbs, originally isolated by Fujita *et al.* (9) for a study of the *Drosophila melanogaster* nervous system, we tested for reactions with the human central nervous system. A surprisingly large number of cross-reactions showing a wide variety of specificities was observed. In addition to providing new markers for the human nervous system, the molecular characteristics of these antigens are of interest in respect to their evolutionary conservation and their possible roles in development, behavior, and disease.

MATERIALS AND METHODS

Generation of MAbs. The mouse hybridomas were generated by using *Drosophila melanogaster* head, brain, or retina homogenates as immunogens, fusing spleen cells with NS-1 myeloma cells, and cloning by limiting dilution. Supernatant fluids were screened on frozen sections of fly heads by indirect immunofluorescence microscopy (9).

Immunohistochemistry. Fresh human central nervous system tissue was obtained postmortem from four patients, age 21–42 years, with no known neurological diseases. Samples were dissected from spinal cord, optic nerve, hippocampus, cerebellum, lymph node, and liver. Blocks of approximately 1 cm^3 were rapidly frozen in isopentane chilled with liquid nitrogen and stored at −90°C. Cryostat sections (8 μm) were placed on multiwell slides (Roboz, South Pasadena, CA) and stored at −20°C for 1 week or less. Staining was at room temperature. Each section was covered with 50 μl of hybridoma supernatant diluted 1:3 with phosphate-buffered saline (P_i/NaCl: 0.26 M KCl/0.14 M NaCl/0.25 M Na_2HPO_4/0.15 M KH_2PO_4, pH 7.4) in a humidified chamber for 30 min, rinsed twice for 5 min in P_i/NaCl, and incubated 15 min with 50–100 μl of fluorescein isothiocyanate-conjugated goat anti-mouse IgG (heavy and light chains) (Cappel Laboratories, Cochranville, PA) diluted 1:50 with P_i/NaCl. After a final 5-min rinse in P_i/NaCl, the sections were coverslipped with 90% (vol/vol) glycerol in P_i/NaCl, containing paraphenylenediamine (1 mg/ml) to reduce bleaching (10), and were viewed with a Zeiss epifluorescence microscope.

Immunoblots. Tissue homogenates in gel electrophoresis sample buffer [2.3% sodium dodecyl sulfate/5% mercaptoethanol/63 mM Tris·HCl, pH 6.8/10% (vol/vol) glycerol] were prepared in a Dounce glass homogenizer. For each gel, the sample was either human central nervous system tissue (18 mg of protein in 200 μl of sample buffer) or 60 fly heads (6 mg of protein) in 100 μl of sample buffer. The samples were electrophoresed in 10% polyacrylamide gel in Tris/glycine buffer (11). The gels were electroblotted to nitrocellulose paper with 192 mM glycine/25 mM Tris base/20% methanol (12). Strips (3-mm wide) were cut and incubated overnight at room temperature with 3 ml of 1% bovine serum albumin (Sigma, RIA grade) in buffer A (0.15 M NaCl/10 mM Tris·HCl, pH 7.4/0.025% Nonidet P-40). Each strip was rinsed with buffer A and incubated overnight at 22°C with 1 ml of a hybridoma supernatant diluted to 3 ml with buffer A, washed three times with buffer A, and incubated for 2 hr with 3 ml of buffer A containing 9×10^5 cpm per strip of ^{125}I-labeled sheep antimouse IgG (heavy and light chains) (Amersham; specific activity, 5 μCi/μg; 1 Ci = 37 GBq). The strips were washed three times with buffer A and once with distilled water, air dried, and autoradiographed.

RESULTS

Tissue Specificities of MAbs. Many of the hybridoma clones obtained with *Drosophila* tissue as primary immunogen produced MAbs that were cross-reactive with the human central nervous system. Of 146 MAbs tested, 69 showed immunofluorescent staining in one or more of the human nervous system sites tested. Tissue samples from three or more different individuals were tested for each site and for each MAb and gave consistent results. Table 1 shows the number of positive reactions for each central nervous system region. Many of the MAbs stained comparable structures in all regions, while some were specific to one.

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Abbreviation: MAbs, monoclonal antibodies.

FIG. 1.

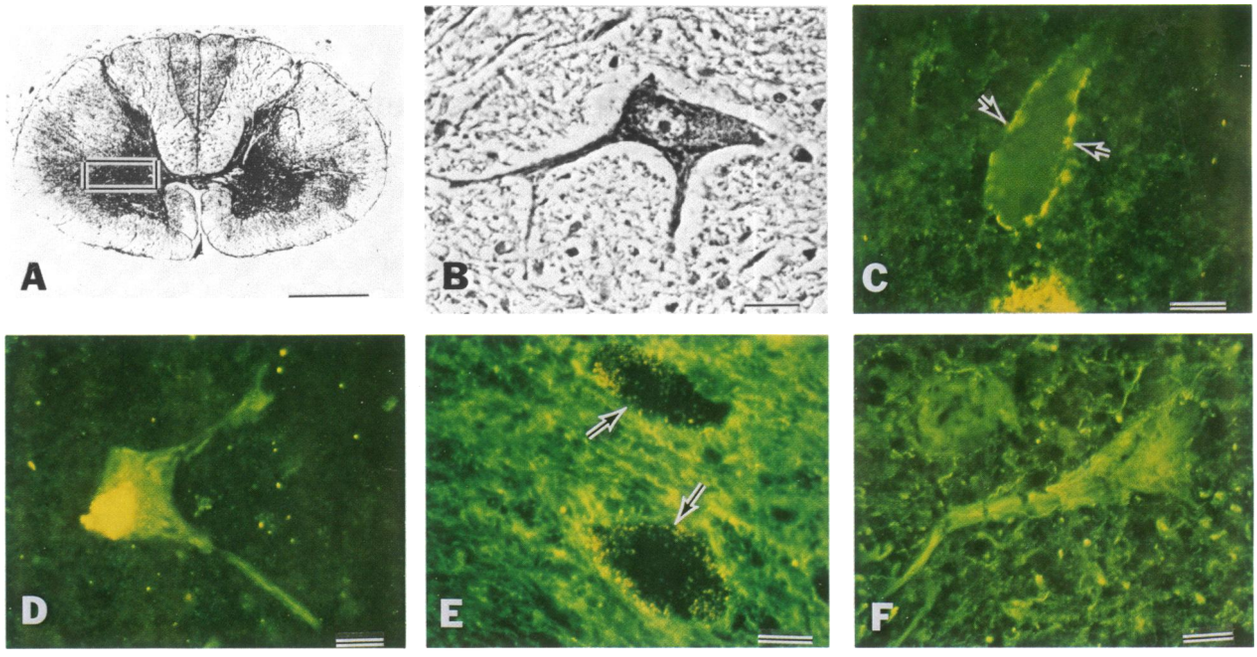

FIG. 2.

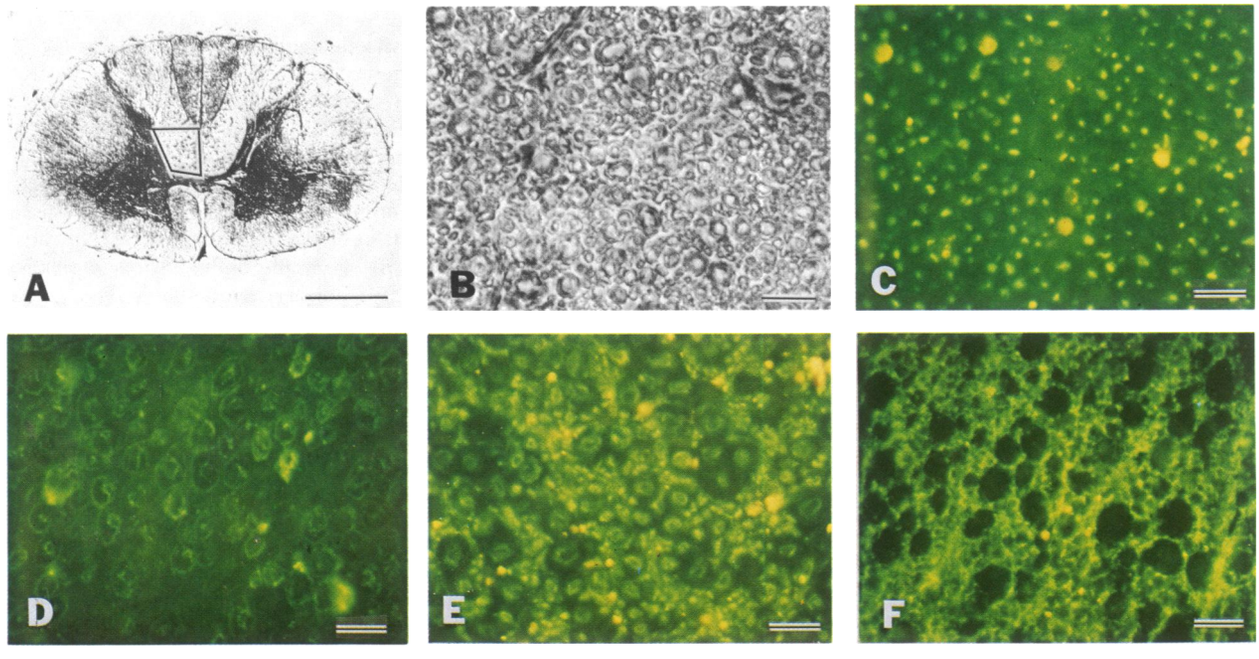

FIG. 1. Spinal cord: motor neurons of the anterior horn. (*A*) Cross section through normal thoracic cord (Bodian stain). Enclosure indicates region in subsequent micrographs. (Bar = 200 μm.) (*B*) Typical anterior horn cell, as seen by phase microscopy. (*C*) MAb 6B5 shows prominent staining at cytoplasmic membrane of perikaryon of anterior horn cell (arrows). (*D*) MAb 13H11 reacts with cytoplasm of anterior horn cell. (*E*) MAb 3G12 stains neuropil, but perikarya of motor neurons (arrows) are unstained. (*F*) MAb D12B reacts with fibrillar material within the perikaryon and also the adjacent neuropil. (Bars in *B–F* = 20 μm.) Bright yellow stain in this and other figures is autofluorescence of cytoplasmic lipofuchsin.

FIG. 2. Spinal cord: dorsal column nerve tracts. (*A*) Enclosure indicates region examined. (Bar = 200 μm.) (*B*) Phase micrograph shows cross section through axons of varying diameters. Myelin appears dark. (*C*) MAb 3F12 stains central regions of axons. (*D*) MAb6B5 stains concentric circles, apparently axonal membranes and myelin sheath peripheries. Larger axons stain more intensely. (*E*) With MAb 8C3, myelin sheaths appear unstained, whereas axonal membranes are stained. (*F*) MAb 3G12 reacts with matrix between nerve fibers, but fibers themselves are unstained. (Bars in *B–F* = 20 μm.)

FIG. 3 (*on next page*). Cerebellar cortex. In immunofluorescence micrographs, internal granule cell layer is to the left, molecular layer is to the right. Arrows indicate Purkinje cells. (*A*) MAb G12 shows a Purkinje cell enwrapped with processes characteristic of basket fibers. (*B*) With MAb 23H2, Purkinje cell and internal granule cells are outlined by punctate halos. (*C*) MAb 8C3 reacts with neuropil only. Neuronal perikarya of Purkinje and internal granule neurons are unstained. (*D*) MAb 10G4 stains Purkinje cell cytoplasm but not nucleus. (*E*) MAb 3F7 selectively stains nuclei (but not nucleoli) of Purkinje and internal granule cells. (*F*) MAb 12E4 stains nuclear membranes of Purkinje cell and internal granule cells. (Bars = 20 μm.)

FIG. 3.

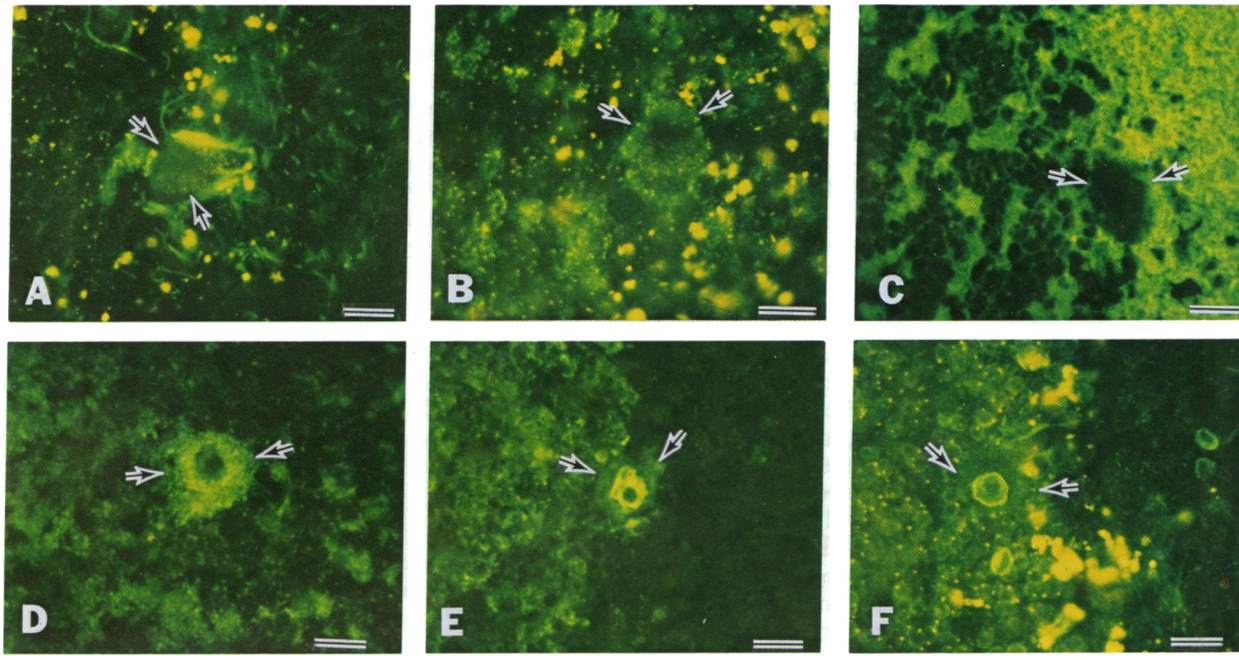

FIG. 4.

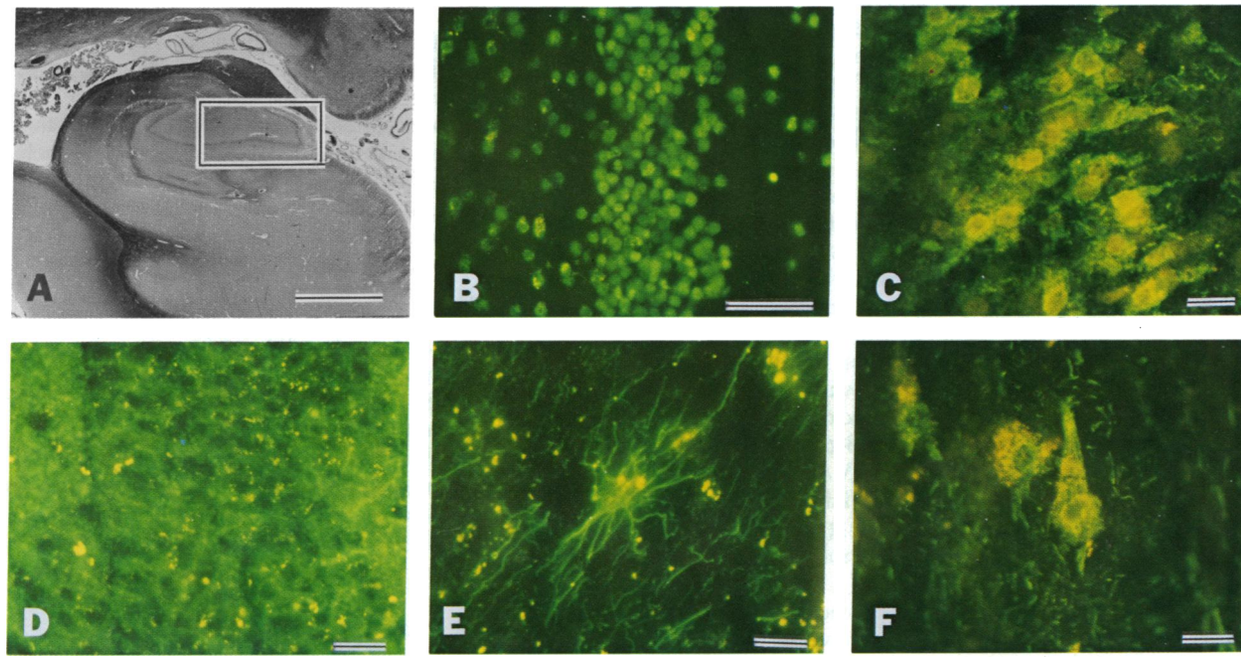

FIG. 5.

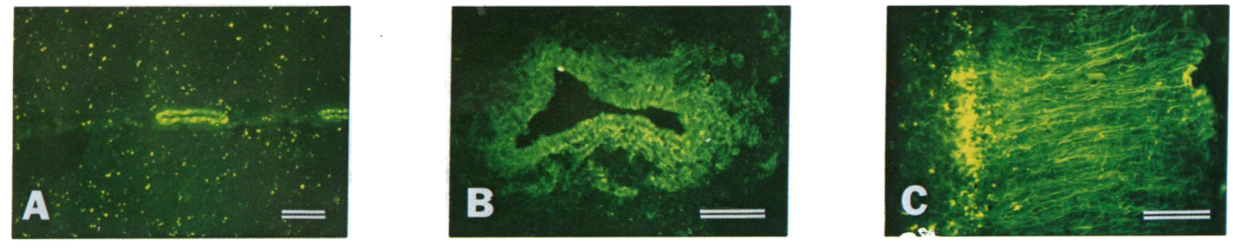

FIG. 4. Hippocampus. (*A*) Section at the level of the lateral geniculate nucleus, showing small neurons of the dentate gyrus, and pyramidal cells. Luxol fast blue/cresyl violet stain. Immunofluorescence microscopy is on the area designated by enclosure. (Bar = 200 μm.) (*B*) MAb 8C5 stains all nuclei, illustrating cells of dentate gyrus. (*C*) MAb 2F12 selectively stains neurons of the dentate gyrus. (*D*) MAb 10G9 stains neuropil but not the dentate gyrus cells. (*E*) MAb 6E1 stains a stellate cell. (*F*) MAb 3F12 stains pyramidal neurons and their processes. (Bars in *B–F* = 20 μm.) Cytoplasm in cells of *C* and *F* contains lipofuchsin (yellow) in addition to antigen.

FIG. 5. Glial and vascular specificity. Section through spinal cord shows selective staining of small arteries (MAb 3H3) (*A*) and ependyma and subependyma of central canal area (MAb 12D12) (*B*). (*C*) Cerebellar cortex is traversed by fibrils characteristic of Bergman's astrocytes (MAb 14F5). (Bars = 100 μm.)

A wide diversity of staining patterns was observed, with relatively little redundancy. Antigens were often specific to one cell class or subcellular component. Sixteen MAbs showed distinct staining of portions of neurons. Four MAbs reacted strongly with neuropil but did not stain neuronal cell bodies, and four stained axons alone. In addition, five MAbs stained cell nuclei in all tissues examined. One MAb (12E4) was specific to the membranes of neuronal cell nuclei.

Fig. 1 shows examples of motor neurons staining in the anterior horn of the spinal cord. MAb 6B5 selectively stained focal areas on the motor neuron plasma membrane, possibly identifying synaptic terminals on the perikaryon. MAb 13H11 stained the cytoplasm of the neuron, whereas MAb 3G12 was positive for the neuropil, leaving the perikarya as "black holes." MAb D12B reacted with both neuropil and perikaryon.

Microscopic detail of axonal staining was observed in transverse sections of the dorsal column of the spinal cord (Fig. 2). Various MAbs distinguished among the components of the axons and their myelin sheaths. MAb 3G12 stained the matrix between nerve fibers, leaving the fibers themselves unstained.

Subcellular staining differences also could be defined within the cerebellum. An antigenic dissection of the Purkinje cell is illustrated in Fig. 3. MAb G12 revealed processes enwrapping the cell in a pattern characteristic of basket fibers. MAb 23H2 outlined the Purkinje cells (and also the internal granule cells) with punctate halos. MAb 10G4 stained cytoplasm, but not nucleus; MAb 3F7 stained nucleus, but not nucleolus. MAb 12E4 stained only the nuclear membranes of the Purkinje cells (and also internal granule cells). An example of negative specificity was shown by MAb 8C3, which stained neuropil, the neuronal perikarya appearing as blank areas.

Fig. 4 illustrates specificity within the hippocampus. The various MAbs stained neurons of the dentate gyrus, pyramidal neurons and their processes, stellate cells, nuclei, or neuropil.

Eight MAbs showed specificity for cells judged to be astrocytes by their shapes and locations in phase-contrast and hematoxylin/eosin-stained sections. Three other MAbs stained apparent oligodendrocytes. Verification of cell class will require immunoperoxidase localization with known molecular markers, such as anti-glial fibrillary acidic protein for astrocytes, anti-myelin basic protein and β-galactocerebroside for oligodendroglia. As seen in Fig. 5*A*, MAb 14F5 revealed fibrillar processes characteristic of the infrastructure of Bergman's astrocytes in the cerebellum. Fig. 5*B* shows the subependymal astroglial matrix and ependymal cells stained by MAb 12D12.

Two MAbs reacted exclusively with blood vessels [e.g., MAb 3H3 (Fig. 5*C*)], which stained the entire vessel wall. Other antibodies, although staining some nonvascular structures as well, reacted with selected portions of the vascular wall, such as the luminal surfaces of endothelial cells, the media, or the adventitia.

A large number of antibodies, not illustrated here, reacted with various combinations of cell types. Important molecular relationships between them may emerge when the antigens are identified.

Table 1. Human central nervous system specificities of monoclonal antibodies

Region	MAbs tested		
	Total no.	No. positive for region	No. unique to region
Spinal cord	146	46	2
Optic nerve	146	31	3
Hippocampus	146	41	7
Cerebellum	146	48	7
Lymph node	32	2	0
Liver	32	2	0

The majority of antibodies manifested strong specificity for the nervous system. Thirty-two of the strongly neuroreactive antibodies were selected to be tested on human liver and lymph node. Only two were positive (Table 1). They stained cytoplasm in both tissues. Another set of five MAbs that stained the cell nuclei in all central nervous system tissues was also tested. They stained liver and lymph node nuclei as well.

Immunoblot Analysis. Antigens identified by 23 neuroreactive MAbs were examined. Some blots showed single bands and others showed several bands or a continuum of radiolabeled material, the latter suggestive of proteins modified by lipid or carbohydrate groups. In some cases, the patterns for *Drosophila* and human antigens showed close similarities. Fig. 6 shows three such pairs.

DISCUSSION

MAbs provide an incisive method for mapping the molecular anatomy of the human nervous system. They can greatly extend the molecular repertoire beyond the relatively limited number of known neuro-specific markers. Unlike conventional histological stains, where the molecular specificity may be elusive, MAbs can be used to purify and identify the antigens.

The remarkably high degree of cross-reactivity between fly and human brain and the occurrence of similar patterns on immunoblots suggests the possibility of evolutionary conservation of many more central nervous system molecules than hitherto known. The occurrence of a cross-reaction does not, by itself, demonstrate homology in primary protein structure. In any particular case, the MAb could be reacting with similar epitopes in otherwise quite different molecules. The epitopes could be in protein, carbohydrate, or lipid moieties. This question will be addressed by identifying the reactive group of the antigen in each species and, where the protein moiety is involved, by peptide analysis. It will be of interest to learn whether the antibodies indeed identify molecules that are common to nervous systems that have evolved so differently in the invertebrate and vertebrate branches.

Our results were surprising because, as a rule, MAbs show narrower species range than do polyclonal antibodies. For instance, polyclonal antibody to myelin basic protein cross-reacted with many mammalian species, whereas MAbs were specific for subgroups (13).

Cross-reactivity between vertebrate species has been documented. Using human central nervous system tissue as primary immunogen, McKenzie *et al.* (14) found a MAb, reactive with a brain glycoprotein, that cross-reacted with the central nervous system of rat, dog, and mouse but not of frog or chicken. Barnstable (15) found MAbs to rat retina that cross-reacted with tiger salamander retina. Others have shown MAb cross-reactions of rat cerebellar antigens with central nervous system of mouse, rabbit, sheep, pig, cow, and human (16, 17). Cross-reactivity of a chicken central nervous system MAb with mouse, rat, and rabbit has been identified (18). Using hamster central nervous system tissue as primary immunogen, Franko *et al.* (5) showed cross-reactivity with human central nervous system in 19 of 30 MAbs. Only 3 were neuron-specific in hamsters and none in human.

All these cases of cross-reactivity involved vertebrates. Relatively few comparisons have been made between species as disparate as human and fly, perhaps because such homologies were expected to be rare. Nevertheless, bizarre examples occur, such as a MAb to horseradish peroxidase showing a cross-

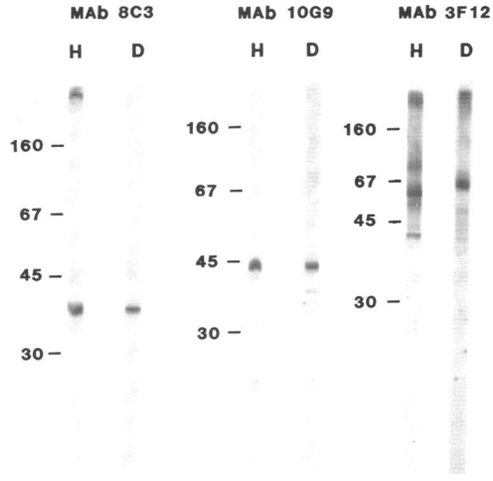

FIG. 6. Immunoblot analysis of human and *Drosophila* antigens using *Drosophila* MAbs. Homogenates of human spinal cord (columns H) or *Drosophila* heads (columns D) were electrophoresed on polyacrylamide gels (10% for MAbs 8C3 and 10G9, 12% for MAb 3F12) and electroblotted to nitrocellulose. The nitrocellulose was cut into strips and each strip was stained with the specified MAb. Autoradiograms showed binding of ^{125}I-labeled second antibody. Molecular weight markers: carbonic anhydrase, 30 kilodaltons (kDa); ovalbumin, 45 kDa; bovine serum albumin, 67 kDa; *Escherichia coli* RNA polymerase β subunit, 160 kDa; jack bean urease, 240-kDa. For each MAb, human central nervous system and *Drosophila* head samples were run simultaneously in parallel gels.

reaction to *Drosophila* axons (19). Our results suggest that it may be worthwhile to scan more boldly between species.

The immunofluorescence screening method, using sections, is relatively rapid and sensitive. Detection of only a few reactive cells is feasible, and the method provides a direct localization of the antigen in tissue. Other immunoassays, while highly sensitive and appropriate to isolation and quantification of antibodies to an already purified antigen, do not offer these advantages for detecting unknown and possibly rare molecules unique to specific structures.

One problem with human tissue is the postmortem delay in obtaining samples; autolysis, with alteration of the antigens, may occur. Whereas our experience with tissues obtained at 6, 12, and 18 hr postmortem indicated no major progressive loss of the antigens observed, shorter postmortem intervals did result in greater preservation of cellular detail. A critical factor was the "snap-freezing" in chilled isopentane. The results reported were for unfixed cryostat sections. Formaldehyde fixation eliminated the staining in all cases. Acetone, on the other hand, had a less destructive effect, with some antigens retaining their activity. Presumably, each antigen will have its own optimal fixation procedure.

In various neurologic diseases, there is selective vulnerability of certain neurons or glial cells. For example, in amyotrophic lateral sclerosis, motor neurons degenerate; in Huntington's disease, cells of the caudate nucleus deteriorate; in some cases of Alzheimer's disease, cells in certain basal forebrain nuclei are lost (20). MAbs may be useful as tags for sorting of specific cell types to identify the molecular profiles of such selectively vulnerable cells and to detect missing or novel antigens in diseased tissue.

Many human neurological defects are hereditary, but progress in human disease has been stymied often by the lack of model systems. *Drosophila* mutants also display hereditary pathologies such as brain degeneration in the *drop dead* mutant (21) and muscle defects resembling nemaline myopathy in the *wings-up* mutant (22). With currently available recombinant DNA technology, it may well be feasible to transfer a selected gene from human to fly in order to study its function. Complications in this procedure arise because, due to the existence of introns and degeneracy in the genetic code, the DNA structures in each organism may differ considerably, even if their protein products are closely homologous. Therefore, the molecular homology revealed by a MAb could be crucial. In principle, it makes possible the isolation of the mRNA from polysomes of each organism, hence, leading to cDNA probes for the isolation of their respective genes.

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