APPLICATIONS OF MONOCLONAL ANTIBODIES TO NEUROSCIENCE RESEARCH

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INTRODUCTION

The introduction of immunological methods has revolutionized neurobiology. Anatomical studies have profited from immunocytochemical localization of neurotransmitters and peptides for a better understanding of the organization of the nervous system. Antibodies have also been useful in identifying different cell classes in the nervous system, and antibodies to subcellular organelles have contributed to our knowledge of neuron function. Monoclonal antibodies are increasingly used by neurobiologists to build on this knowledge.

This review consists of four major sections. In the first three, applications of monoclonal antibodies are reviewed with regard to the cell biology of the neuron and the anatomy and development of the nervous system. The final section is an overview of the strategies involved in isolating and applying monoclonal antibodies to studies of the nervous system. This is not intended to be a comprehensive review of the literature of these subjects, and, where possible, reviews of individual subjects are indicated in the text.

In some areas, such as peptide and transmitter research, the main contributions of monoclonal antibodies have been to make scarce antibodies more widely available and to make antibodies to antigens that have been difficult to purify. In developmental neurobiology, monoclonal antibodies have made a promising beginning in identifying molecules that might be involved in cell-cell recognition and cell adhesion. Some of the most interesting contributions of monoclonal antibodies have been in determining protein structure (e.g. acetylcholine receptor and sodium channel), and we discuss these areas in greater detail.
NEURONAL CELL STRUCTURE AND FUNCTION

Channels, Pumps, and Receptors

SODIUM CHANNEL  Voltage sensitive Na⁺ channels play a key role in conducting the action potential and are the one channel type that has been significantly purified and studied with traditional biochemical methods (reviewed in Reichardt & Kelly 1983). The Na⁺ channel has been purified substantially from Electrophorus electroplax, rat brain, and rat muscle. Purified channels from the latter two preparations have been successfully reconstituted in lipid bilayers. All channel preparations contain a very large (ca 270 kDa) glycoprotein. Smaller subunits have been seen in the purified channel preparations from rat brain and rat muscle. During the last two years, conventional antisera and monoclonal antibodies have been raised that bind the large glycoprotein subunit of the channel, both from Electrophorus electroplax and rat muscle (Moore et al 1982, Ellisman & Levinson 1982, Casadei et al 1983). A monoclonal antibody that binds the Electrophorus channel subunit has been used to complete the purification of that subunit by affinity chromatography (Nakayama et al 1982). Another monoclonal antibody has shown that Na⁺ channels are concentrated on the electrically excitable caudal face of the electrocytes and not on the inexcitable rostral face (Fritz & Brockes 1983). There was no detectable binding with this antibody to sections of Electrophorus spinal cord, muscle, or nerve, or to electrically excitable tissues of other species. In contrast, a polyclonal antiserum to a highly but not completely purified Electrophorus Na⁺ channel preparation binds to both the innervated surfaces of electrocytes and to nodal regions in myelinated axons (Ellisman & Levinson 1982). The monoclonal antibody appears to recognize an antigenic determinant found only on the Na⁺ channel in the electroplax. The polyclonal serum appears to recognize a more widely shared determinant. The specificity of the polyclonal antiserum for the Na⁺ channel, however, has not been assessed using total membrane proteins. The distribution of Na⁺ channels in dendrites, axons, and nerve terminals is functionally important in modulating integration of different synaptic inputs, initiation and conduction of the action potential, and neurotransmitter release. Detailed studies using these antibodies and antibodies to other channels will be very important in understanding the electrical properties of excitable cells. Monoclonal antibodies to the small Na⁺ channel subunits should be useful in understanding the role of these subunits in Na⁺ channel function.

SODIUM PUMPS  The major mechanism for removing internal Na⁺ and restoring internal K⁺ after action potentials is the ATP driven Na⁺-K⁺ exchange pump. It is detected in biochemical assays as a Na⁺, K⁺-ATPase, which contains two polypeptide subunits of approximate Mr 120,000 and 50,000.
Distribution of the Na\(^+\), K\(^+\)-ATPase on neurons in fish brain has been examined with immunocytochemical methods using a conventional antiserum (Wood et al 1977). On neurons, it is restricted to the plasmalemma, where it is distributed over the surface of cell somata and dendrites. It appears to be concentrated in nodes of Ranvier in myelinated axons. Two classes of synaptic terminals were observed, one with high and one with lower concentrations of the ATPase. Differences in concentration between different nerve terminals are likely to be important in regulating the response properties of these terminals to stimulation.

There is biochemical evidence for multiple forms of the Na\(^+\), K\(^+\)-ATPase in neurons (Sweedner 1979). One form is the standard form (α) that is seen in kidney, muscle, and astrocytes in addition to cultured sympathetic neurons. A specific neuronal form (α\(^+\)) is found in both vertebrate and invertebrate brain and is the only form found in myelinated axons. Recently, a monoclonal antibody to the Na\(^+\), K\(^+\)-ATPase has been isolated that suggests the existence of an even wider diversity of Na\(^+\)-pumps (Fambrough & Bayne 1983). This antibody binds the Na\(^+\), K\(^+\)-ATPase in chicken neurons, muscle fibers, and kidney tubules, but not in Schwann cells, fibroblasts, or erythrocytes. This distribution is not the same as that of α\(^+\) in rodent tissues. The antibody binds to nonglycosylated antigen, so the heterogeneity revealed by the antibody is unlikely to reflect posttranslational modification. The most likely explanation is molecular heterogeneity, arising either from differential expression of members of a closely related gene family or differences in subunit composition in different cell types. The monoclonal antibody has been used to study regulation of Na\(^+\) pump levels in response to demand for pumping and to study distribution of the pump in chick neurons (Fambrough 1983). In contrast to fish brain, substantial concentrations of the Na\(^+\) pump appear to exist in the internodal regions of chick myelinated axons.

Ca\(^{2+}\)-PUMPS Cytoplasmic Ca\(^{2+}\) levels regulate many facets of neuronal metabolism, most notably the release of neurotransmitters by exocytosis. Cytoplasmic Ca\(^{2+}\) levels are regulated by the activity of voltage-sensitive Ca\(^{2+}\) channels and several Ca\(^{2+}\) removal systems, including a Na\(^+\):Ca\(^{2+}\) antiporter in the plasma membrane, a Ca\(^{2+}\) porter in the mitochondrial inner membrane, and several distinct ATP-dependent Ca\(^{2+}\) uptake systems in the plasma membrane, smooth endoplasmic reticulum, and synaptic vesicles (reviewed in Reichardt & Kelly 1983). The major neuronal ATP-dependent Ca\(^{2+}\) translocators have been purified (Goldin et al 1983, Chan et al 1984). An Mr\(^+\) 140,000 translocator, regulated by Ca\(^{2+}\)-calmodulin, has the same molecular weight as the Ca\(^{2+}\)-calmodulin-regulated (Ca\(^{2+}\) + Mg\(^{2+}\)) ATPases in red blood cells and many other tissues. A second, Mr\(^+\) 94,000 Ca\(^{2+}\) translocator is not regulated by Ca\(^{2+}\)-p-calmodulin and is similar in size to the Ca\(^{2+}\)
translocator in muscle sarcoplasmic reticulum. A monoclonal antibody to this neuronal translocator, however, does not cross-react with either the sarcoplasmic reticulum or red blood cell translocators (Chan et al. 1984), thus suggesting that this is a nerve-cell-specific protein. The antibodies to this translocator should facilitate studies on the regulation of its activity. Its ultrastructural localization will also be very interesting.

ACETYLCHOLINE RECEPTOR The acetylcholine (ACh) receptor has been intensely studied with monoclonal antibodies, yielding much information about structure and function. The ACh receptor is a pentameric protein complex that forms a transmembrane channel that is opened by binding to ACh (reviewed in Conti-Troncini & Raftery 1982). The receptor consists of four different, but homologous, subunits that form a pentameric rosette around a central channel with stoichiometry \( \alpha_2\beta_2\gamma_2\delta \). It is the primary target in myasthenia gravis and in an animal disease model, experimental autoimmune myasthenia gravis (EAMG).

Several groups have isolated monoclonal antibodies to the ACh receptor, purified from *Torpedo* electroplax, *Electrophorus* electroplax, or mammalian skeletal muscle (Mochley-Rosen et al. 1979, Tzartos & Lindstrom 1980, Conti-Troncini et al. 1981). Monoclonal antibodies that bind determinants unique for each subunit have been identified (Tzartos & Lindstrom 1980). Among these are a few examples of antibodies that block ion flux and snake \( \alpha \)-toxin binding (Mochley-Rosen et al. 1979, Gomez et al. 1979). Antibodies that recognize homologous determinants shared by the \( \alpha \) and \( \beta \), or \( \gamma \) and \( \delta \) subunits, respectively, have also been isolated and provide evidence for homologies between these subunits (Tzartos & Lindstrom 1980). Previous studies using conventional antisera and comparative peptide analysis had not revealed these subunit homologies, which have since been confirmed by sequencing completely the four genes that encode these subunits (e.g. Noda et al. 1983). Nine antigenic determinants, some sequence dependent and some conformation dependent, have been recognized on the *Electrophorus* receptor (Tzartos et al. 1981). Twenty-eight determinants have been recognized on denatured *Torpedo* receptor subunits (Gullick & Lindstrom 1983). Fab fragments of an antibody that binds the \( \alpha \)-subunit have been used in high resolution electron-microscopic studies to map the position of the \( \alpha \)-subunits in *Torpedo* receptor dimers in which the \( \delta \)-subunits link the two monomers (Kristofferson et al. 1984). The results show clearly that the two \( \alpha \)-subunits are not adjacent to each other, but are separated by an intercalated \( \beta \) or \( \gamma \)-subunit. Examination of the binding of Fab fragments specific for other subunits should reveal the order in which the subunits surround the central ion channel. Antibodies that bind to different antigenic determinants are being used to probe the structure of each receptor subunit. In many cases these have been shown to bind different peptides (Gullick et al. 1981, Gullick & Lindstrom 1983). Some, but not all, of the
epitopes in the δ- and β-subunits have been ordered along the peptide sequence by examining the sensitivity to limited proteolysis of in vitro synthesized subunits that have been co-translationally inserted into microsomes (Anderson et al 1983). Extracellular and cytoplasmic domains on the β subunit have been identified. In general, these results are consistent with models of subunit structure derived from hydrophobicity of the amino acid sequence, but are not sufficiently refined to determine whether a fifth amphipathic α-helix crosses the lipid bilayer (Finer-Moore & Stroud 1984). Four and five transmembrane domain models of the acetylcholine receptor could in theory be distinguished by determining whether antibodies specific for the C-terminus of a receptor subunit bind to the cytoplasmic or extracellular surface. Recently, an antibody to a synthetic peptide corresponding to the C-terminus of the δ-subunit has been shown to bind to the cytoplasmic side of the membrane, arguing persuasively for a five trans-membrane domain model (Young et al 1984).

The acetylcholine receptor contains a major immunogenic determinant that is located on the extracellular surface of the α-subunit and is conserved between species (Tzartos & Lindstrom 1980; Tzartos et al 1981). Antibodies to this determinant do not prevent ion flux through the channel, but do induce passive EAMG when injected into rats (Tzartos et al 1981). The ability of different monoclonal antibodies to induce EAMG has shown to correlate with their ability to aggregate solubilized receptor in vitro (Conti-Troncini et al 1981). As expected, only antibodies to the α subunit, two copies of which are included in each receptor, are able to aggregate receptor into complexes larger than dimers. Only those anti-α antibodies that induce the formation of large aggregates efficiently induce EAMG. These antibodies are also the only ones to increase the turnover of ACh receptor on the surface of cultured myotubes. Monoclonal antibodies have been used in competitive binding assays to determine the specificity of anti-receptor antibodies in the serum of patients with myasthenia gravis. Patients with myasthenia gravis produce antibodies to the same regions on receptor as produced by animals immunized with receptor purified from fish electric organ (Tzartos et al 1982). Most of these bind to the main immunogenic region. These results suggest that antibody production in myasthenia gravis is stimulated by receptor, not by a cross-reacting antigen. No correlation was found between the titer of antibody to a specific determinant and the severity of the disease state. This suggests that other factors are important in determining the severity of illness. Monoclonal antibodies will make it possible to investigate these factors in detail.

RECEPTORS TO OTHER LIGANDS Monoclonal antibodies to the β-adrenergic receptor have been isolated and used to map its distribution in the brain, where it is concentrated in postsynaptic densities (Strader et al 1983). Monoclonal antibodies to purified rat brain muscarinic acetylcholine receptor have been
isolated and have been used to show that all identified antigenic epitopes on these receptors are conserved in all tissues and species examined, including both vertebrates and invertebrates (Ventner et al 1984). These data suggest that there is only one form of the receptor and that it has been extraordinarily conserved throughout evolution. Monoclonal antibodies to the α-adrenergic receptor have been isolated by immunizing with rat liver plasma membranes (Ventner et al 1984). Some of these antibodies cross-react with the muscarinic acetylcholine receptor. The receptors may share antigenic sites because they modulate common effectors. These results show that plasma membrane preparations can be used to obtain monoclonal antibodies specific for receptors, provided that the antibodies can be screened effectively. Plasma membranes have been used to obtain monoclonal antibodies specific for both the epidermal and nerve growth factor receptors (Richert et al 1983, Chandler et al 1984).

Internal Organelles and Cytoskeletal Elements

The complex subcellular anatomy of neurons has been examined with conventional and monoclonal antibodies. The studies have revealed that specific molecules are concentrated in anatomically distinct regions of the cell.

CYTOSKELETAL ELEMENTS Neurons contain the same cytoskeletal elements as other cells—actin, tubulin, and intermediate filament networks. The role of these filaments in neural function is poorly understood, although fast transport of membrane vesicles has been closely associated with microtubules in vivo and in vitro and requires the integrity of the microtubule network (reviewed in Reichardt & Kelly 1983). Studies initiated with conventional antibodies and extended with monoclonal antibodies have examined the distribution of actin, tubulin, and intermediate filament-associated proteins in cultured neurons and in sections of the brain. Antibodies specific for each subunit of the neurofilament triplet have shown that all three subunits are found only in neurons (e.g. Shaw et al 1981a, Lee et al 1982). Some classes of neurons, for example the granule cells in the cerebellum, lack neurofilaments and do not bind antibodies to any of the neurofilament subunits (e.g. Shaw et al 1981b). In electron-microscopic examination, each antibody can be shown to stain the same filament (Sharp et al 1982). The $M_r$ 200,000 subunit appears to have a periodic distribution in the filaments, while the other two smaller subunits are distributed continuously (e.g. Sharp et al 1982). While the three neurofilament subunits always appear to coexist in the same neurons, the $M_r$ 200,000 subunit does not appear to be found in neurofilaments in all parts of some classes of neurons (Hirokawa et al 1984). For example, a monoclonal antibody specific for the $M_r$ 200,000 subunit does not bind to dendrites in pyramidal cells, even though these dendrites have high concentrations of the $M_r$ 68,000 subunit (Debus et al 1982). More recent work has raised the pos-
sibility, though, that some of the observed restrictions in distribution of the $M_r$ 200,000 subunit may reflect differences in its state of phosphorylation. Many monoclonal antibodies to the subunit have been shown to be specific for either phosphorylated or nonphosphorylated forms of the subunit (Goldstein et al 1983, Sternberger & Sternberger 1984). The two classes of antibodies stain different neurons and parts of neurons. Their staining pattern suggests, for example, that the $M_r$ 200,000 subunit is more heavily phosphorylated in axons than in dendrites of Purkinje cells.

A differential distribution of other cytoskeletal proteins has also been shown in pyramidal and Purkinje cells. A conventional antiserum to the high molecular weight microtubule-associated-proteins (MAP1 and MAP2) stains dendrites but not axons, whereas antibodies to tubulin bind to both regions (Matus et al 1981). More recent studies with a MAP2 monoclonal antibody suggest that MAP2 is restricted to dendrites, while MAP1 is probably not restricted in its distribution (Caceres et al 1983, 1984, Vallee & Davis 1983, Huber & Matus 1984). MAP2 has been shown to be a substrate for both cAMP-dependent and Ca$^{2+}$-calmodulin dependent protein kinases, and it has a binding site for the type II regulatory subunit (RII) of the cAMP-dependent kinase. Binding sites for the RII subunit are concentrated in dendrites and are almost indetectable in axons. This is essentially the same distribution reported for MAP2 (Miller et al 1982). A differential distribution of actin and $\beta$-tubulin has also been shown in the Purkinje cell dendritic shaft and dendritic spine. A monoclonal antibody to $\beta$-tubulin stained only the dendritic shaft, whereas antibodies to MAP2 and actin stained both the shaft and the spine (Caceres et al 1983). One monoclonal antibody to actin, however, did not stain actin in the spines. These results suggest that there may be different functional states of actin and that the interactions between cytoskeletal components may vary in different compartments of the cell.

Conventional antibodies to brain-specific and erythrocyte-specific spectrins, proteins that bind to both the membrane and cytoskeleton, have been used to show that both forms are present in neurons, but have different distributions (Lazarides & Nelson 1983a,b, Lazarides et al 1984). The erythrocyte form appears only at a terminal state in differentiation and is restricted to the cell soma and dendrites, while the brain-specific form, also known as fodrin, appears early in neuronal development and is distributed throughout the neuron. It seems likely that the spectrins function to coordinate domains in the neuronal membrane and cytoskeleton.

As illustrated above, monoclonal antibodies have the potential for identifying and probing the function of modified forms of proteins that are associated with particular regions of the neuron. As one example, injection of a monoclonal antibody specific for the tyrosylated form of $\alpha$-tubulin has been shown to have dramatic effects on organelle movement and cytoplasmic organization.
in fibroblasts. This suggests that addition of tyrosine to tubulin may modulate its activity within this cell (Wehland & Willingham 1983). Intriguingly, monoclonal antibodies specific for the tyrosylated and nontyrosylated forms of α-tubulin stain the axons of cerebellar granule cells differently, depending on their age, in patterns that suggest that tyrosine addition to tubulin is reduced as axons mature (Cummings et al 1984). Monoclonal antibodies to different epitopes on actin and calmodulin stain different subcellular structures in neurons and fibroblasts, respectively, suggesting that these proteins interact with different binding proteins in different compartments (Caceres et al 1983, Pardue et al 1983). Thus, monoclonal antibodies make it possible to study the specific interactions of these proteins with other cellular macromolecules. An individual monoclonal antibody, however, cannot be assumed to reveal all the sites to which its antigen is localized.

ORGANELLES Synaptic vesicles and mitochondria are prominent organelles in nerve terminals. A monoclonal antibody directed against a neuron-specific mitochondrial protein has been isolated using rat brain as an immunogen (Hawkes et al 1982a). This antibody binds a Mr 23,000 protein in neuronal mitochondria that is absent from other cell types in the brain. The antigen appears in neurons only after the termination of cell division. This suggests that changes in mitochondrial proteins may be important in neuronal differentiation.

Monoclonal procedures have been very useful for identifying antigens shared by many classes of synaptic vesicles. Two monoclonal antibodies to a Mr 65,000 vesicle membrane protein bind vesicles in many and possibly all types of nerve terminals (Matthew et al 1981). The antibodies have been used successfully to purify synaptic vesicles from crude brain homogenates by immunoprecipitation and to confirm the presence of norepinephrine and several peptide transmitters in vesicle fractions. The vesicle antigen is highly conserved throughout the vertebrate phylogeny. A second vesicle-specific protein, the phosphoprotein synapsin I, was initially studied with polyclonal antibodies and was shown also to exist in virtually all types of nerve terminals and throughout the vertebrate phylogeny (e.g. Goelz et al 1981). More recently, monoclonal antibodies have been isolated to this phosphoprotein and these should make possible more detailed studies on the structure and function of synapsin I in the future (Nestler & Greengard 1983). The distributions of these two antigens have been used to monitor differentiation of the nerve axon and nerve terminal during development in vivo or in vitro. Dramatic changes with development are seen both in the brain and culture dish (Chun & Schatz 1983; Matthew & Reichardt 1982).

Cholinergic synaptic vesicles from fish electric organs contain a specific proteoglycan that has been defined by both conventional and monoclonal anti-
sera (Jones et al. 1982, Buckley et al. 1983). Antibodies have been used to show that this proteoglycan is released into the cleft by exocytosis during stimulation and is recycled during rest (Jones et al. 1982). Studies with a monoclonal antibody show that the antigen is found on the surface of the cholinergic nerve terminals in the vicinity of active zones. Evidence suggests that a fraction remains in the cleft for extended periods during rest, possibly immobilized in the extracellular matrix (Buckley et al. 1983).

**SYNAPSE CONSTITUENTS** Chemical synapses exist in a diversity of morphological conformations, usually containing one or more of the following specializations: presynaptic vesicle clusters, presynaptic membrane densities, and postsynaptic dense material. Conventional and monoclonal antibodies have provided a promising approach to identifying the molecules in the specializations associated with the synapse. Few antibodies to antigens concentrated in the presynaptic nerve terminal have been found so far. The only well-defined antigens are components of synaptic vesicles (discussed under Organelles), which include neurotransmitters and neuropeptides (see next section).

Antibodies to antigens in the postsynaptic specializations are more common. The ACh receptor has been localized at the neuromuscular junction with monoclonal antibodies to the receptor (Z. W. Hall, personal communication), and has also been shown by use of α-bungarotoxin to be in the membrane at the top and sides of the postsynaptic folds (Fertuck & Salpeter 1976). Antibodies to a Mr 43,000 protein that copurifies with ACh receptor have been used to localize the protein at the neuromuscular junction (Froehner et al. 1981). In electron-microscopic examination, the Mr 43,000 protein appears to form a dense bar of contrasted material that is coextensive with ACh receptor (Sealock 1982, Scalock et al. 1984). It can be cross-linked to the acetylcholine receptor with a heterobifunctional reagent. Monoclonal antibodies to the Mr 43,000 protein and four acetylcholine receptor subunits have been used to show that the cross-linked product is a β-receptor subunit-43 kDa dimer (Burden et al. 1983). The same anti-43 kDa protein monoclonal antibody immunoprecipitates a Mr 43,000 protein that binds ATP and has protein kinase activity (Gordon et al. 1983, A. S. Gordon, personal communication), so this monoclonal antibody has made it possible to show rigorously that the Mr 43,000 polypeptide associated with the acetylcholine receptor has protein kinase activity. Concentrated between the folds at the neuromuscular junction are 10 nM filaments, almost certainly intermediate filaments. An amorphous, electron-dense material is between these filaments and the membrane. Recently, a monoclonal antibody has been described that binds an antigen, related antigenically to intermediate filaments, that is concentrated in this amorphous material (Burden 1982). Other antibodies have been used to localize to the neuromuscular junction antigens related to actin and three actin-binding proteins—α-actinin, vinculin, and filamin (Bloch & Hall 1983).
Classical CNS synapses have somewhat similar postsynaptic specializations. Antibodies are not available to most of the receptors in the CNS. Development of monoclonal reagents to these receptors is likely to be one of the major contributions of modern immunology in the next few years. Where receptor antibodies are available, such as to the β-adrenergic receptor, they have localized the receptor to the postsynaptic density (Strader et al 1983). Microfilaments and microtubules have been visualized in the lattice of the postsynaptic density (e.g. Matus 1981). Recently, monoclonal antibodies to β-tubulin, actin, and the high molecular weight microtubule-associated-protein MAP2 have localized these molecules to the postsynaptic density (Caceres et al 1983). The major postsynaptic density protein, a Mr 50 kDa polypeptide of previously unknown function, has recently been shown, by using monoclonal antibodies specific for this kinase (Kennedy et al 1983, Kelly et al 1984), to be the 50 kDa subunit of a Ca\(^{2+}\)-calmodulin-dependent protein kinase. Immunocytochemistry with these antibodies shows that this subunit is not localized exclusively to the postsynaptic density, but is found also in neuronal cell bodies, axons, and terminals in all or nearly all areas of the brain (McGuinness et al 1983). Conventional antisera have been used to demonstrate the presence in the postsynaptic density of a Mr 95,000 protein (Nieto-Sampedro et al 1982), fodrin (Carlin et al 1983), calmodulin (Wood et al 1980), and calcineurin, which is the Ca\(^{2+}\)-calmodulin regulated protein phosphatase 2B (Wood et al 1980). Monoclonal antibodies to these and other antigens will be crucial for delineating the structure and composition of this organelle.

**Extracellular Matrix Components**

The extracellular matrix between the pre- and postsynaptic elements of the neuromuscular junction has become a subject of intense interest. Acetylcholinesterase is inserted into this matrix and is important in removing acetylcholine from the synapse. Acetylcholinesterase occurs in several molecular forms: soluble or membrane bound, intra- or extracellular, with or without a collagen-like tail (reviewed in Massoulie & Bon 1982). The occurrence of forms with a collagen-like tail is correlated with innervation. With the objective of understanding the relationship between the different forms of esterase, Fambrough et al (1982b) isolated five monoclonal antibodies to the plasma-membrane-associated acetylcholinesterase of purified erythrocytes. All of these antibodies recognized an antigen in the neuromuscular junction; two of these were shown to bind to the form of esterase with a collagen-like tail. The results argue that there is a high degree of homology between different forms of the esterase.

It is now clear that components in the synaptic extracellular matrix induce
synaptic specializations in both nerve and muscle during regeneration (reviewed in Burden 1982). Conventional antibodies to anterior lens capsule, two collagen extracts, and a substantially purified factor that induces aggregation of ACh receptors in cultured myotubes have been shown to distinguish synaptic and nonsynaptic regions of basal lamina (Sanes & Hall 1979, Nitkin et al 1983). More recently, antibodies to the major components of basal lamina have been screened for binding specificity (Sanes 1982). Although collagen IV, laminin, and fibronectin are found in both synaptic and nonsynaptic regions, collagen V appears to be excluded from synaptic regions. Monoclonal procedures have been used to generate several antibodies directed to synapase-specific components of the basal lamina (Chiu & Sanes 1982, Fambrough et al 1982a). One antigen has been identified as a heparan sulfate proteoglycan synthesized by aneural muscle cells that is concentrated within the synaptic cleft at the neuromuscular junction (Anderson & Fambrough 1983). This proteoglycan is unlikely to be related to the proteoglycan defined by monoclonal antibodies that is found in synaptic vesicles and on the surface of cholinergic nerve terminals in fish electric organ (Buckley et al 1983). These and other synapse-specific antigens could be important in synapse function, structure, or development.

NERVOUS SYSTEM ANATOMY.

Major Cell Types

One important application that hybridoma technology has had in neurobiology is the generation of probes that recognize different subsets of cells in the nervous system. These range from antibodies that distinguish neurons from glial cells, and peripheral neurons from central neurons, to those which recognize very small subsets of neurons. Antibodies to cell surface antigens are particularly important for isolation of a cell type by positive or negative selection (reviewed by Basch et al 1983). Positive selection procedures include adhesion to antibody columns or magnetic beads (e.g. Meier et al 1982) and cell sorting (Dangl & Herzenberg 1982). Selenium- and iron-conjugated monoclonal antibodies can be used to select antigen-positive cells by growing cell mixtures in defined media that are missing these essential nutrients (Block Bothwell 1983). Antigen-positive cells can also be selected in hydrogen-peroxide-containing media by using peroxidase-conjugated antibodies to protect these cells from the deleterious effects of peroxide (Bach et al 1983). Negative selection procedures include antibody-directed complement- or toxin-mediated killing of unwanted cell types (e.g. Brockes et al 1979, Vitetta et al 1983).

The major cell types in the peripheral and central nervous systems can now be distinguished using conventional antisera. Polyclonal antisera have been produced that distinguish astrocytes, Schwann cells, oligodendrocytes, fibroblasts and neurons (see Table 1 for references). Monoclonal antibodies have
Table 1 Major cell class markers

<table>
<thead>
<tr>
<th>Cell and antigen</th>
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<th>Cell and antigen</th>
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<tr>
<td>Neurons</td>
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<tr>
<td>Tetanus toxin</td>
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<td>Astrocytes</td>
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<td>Neurofilament proteins</td>
<td>2, 3, 4</td>
<td>Glial fibrillary</td>
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<td>5</td>
<td>acidic protein (GFAP)</td>
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<td>N-CAM</td>
<td>6</td>
<td>S-100</td>
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<td>A4</td>
<td>7</td>
<td>α2-Glycoprotein</td>
<td>23</td>
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<td>38/D7</td>
<td>8</td>
<td>Non-neuronal enolase</td>
<td>24</td>
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<td>NILE glycoprotein</td>
<td>9</td>
<td>M1, C1</td>
<td>25, 26</td>
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<td>Ran-2</td>
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<td>Vimentin</td>
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<td>Stage specific embryonic antigen (SSEA-1)</td>
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<td>16</td>
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<td>3</td>
<td>Vimentin</td>
<td>11, 12</td>
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<tr>
<td>(GAL-C)b</td>
<td>18</td>
<td>Fibronectin</td>
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*Shared markers.

been made to some of these cell specific markers, and some new antigens have also been discovered such as M1 and C1 for astrocytes (Lagenaur et al 1980). The astrocyte markers M1 and C1 are expressed at different times during development, with C1 appearing at embryonic day 10 in the mouse. Cell type specific antibodies have been used to study a wide range of other questions, including the requirements for synthesis of myelin components by Schwann cells and oligodendrocytes (Mirsky et al 1980) and the effects of neurons on glial cell differentiation (Holton & Weston 1982).

For the majority of cells, the markers shown in Table 1 are restricted in their distribution. However, some markers are found on more than one cell type, although in some cases distribution of antigen may be altered by artifacts.
of fixation (Ludwin et al. 1976, Ghandour et al. 1981). It has been shown that some Schwann cells may express glial fibrillary acidic protein (GFAP) (Yen & Fields 1981). Also, there is a population of astrocytes in white matter cultures with a neuron-like morphology that expresses two neuronal epitopes: the A2B5 antigen (GQ ganglioside) and tetanus toxin receptor (Raff et al. 1983). These oligodendrocytes and neuron-like astrocytes are derived from a common precursor that is an A2B5-positive, GFAP-negative cell. Culture conditions determine which phenotype is eventually expressed by this precursor (Raff & Miller 1983). These precursor cells have been purified from embryonic optic nerve by using a fluorescence-activated cell sorter to positively select A2B5+ cells (Abney et al. 1983). The purified cells develop into oligodendrocytes in vitro in the absence of living neurons.

**Neuronal Subpopulations**

Conventional and monoclonal antibodies have been used in a wide variety of experiments to detect, categorize, and monitor the development of antigens that distinguish one type of neuron from another. Antibodies that distinguish neuronal subpopulations fall into two classes: (a) antibodies to neurotransmitters, neuropeptides, or enzymes involved in their metabolism; (b) monoclonal antibodies to random antigens identified by screening with immunocytochemical procedures supernatants from large numbers of hybridoma clones.

**Neurotransmitters, Neuropeptides, and Transmitter Enzymes**

The reagents most widely used to distinguish different neurons have been antibodies to neurotransmitters. During the past decade, immunocytochemical examination has shown that more than 30 peptides are localized within specific neurons, coexisting in many cells with other transmitters (e.g. Hökfelt et al. 1980, Iversen 1983). Even larger numbers of peptides have been revealed within the genes for these, and tissue-specific RNA splicing and prohormone processing have further expanded the numbers of possible peptide transmitters. Antibodies capable of distinguishing between related peptides should be particularly valuable for future work. Recently, it has become possible to visualize several classical transmitters directly with antibodies raised to serotonin, glutamic acid, and γ-amino butyric acid coupled to protein carriers (Cuello et al. 1982, Storm-Mathisen et al. 1983, Seguela et al. 1984). These classical transmitters have also been found to be localized within specific subpopulations of neurons.

The number of available monoclonal antibodies to neurotransmitters, neuropeptides, and transmitter-related enzymes is expanding rapidly. Monoclonal antibodies are proving particularly useful in making reagents of defined specificity available in large quantities to many laboratories. We discuss only a few examples of special interest.
Monoclonal antibodies specific for substance P and serotonin have been used in a number of creative applications of monoclonal technology. They have been internally labelled by growth of the hybridomas in the presence of $[^3H]$-lysine. These internally labelled antibodies have been used to localize each transmitter in nerve terminals by high resolution autoradiography (Cuello et al 1982). Internally labelled antibodies offer a number of advantages in immunocytochemistry. First, the antibodies are not chemically coupled and are fully active. Second, molecules as small as $F_{ab}$ fragments can be used to penetrate sections, so it is possible to use these antibody fragments with tissues that are more thoroughly fixed for good preservation of ultrastructure than is possible with antibody conjugates. It is also possible to combine autoradiography and immunoperoxidase to detect two antigens simultaneously using the electron microscope. These procedures have been used to investigate the coexistence of two putative transmitters in individual neurons, e.g. substance P and enkephalin-containing terminals in the substantia gelatinosa (Cuello et al 1982), and substance P and serotonin in the nucleus raphe magnus of the rat (Cuello et al 1982).

Another new method utilizes bispecific antibodies in which one antigen-binding site is directed against a neurotransmitter and the other is directed against horseradish peroxidase (Milstein & Cuello 1983). These antibodies are synthesized in cell lines derived from the fusion of transmitter-specific and peroxidase-specific hybridomas. Their use minimizes the size of reagents that must diffuse into tissue sections.

Monoclonal antibodies specific for several neurotransmitter enzymes have been isolated, including tyrosine hydroxylase, dopamine-$\beta$-hydroxylase, and choline acetyltransferase (CAT) (Ross et al 1981a,b, Crawford et al 1982, Levey et al 1983, Eckenstein & Thoenen 1982). The generation of monoclonal antibodies to the latter enzyme has been particularly useful, since CAT is difficult to purify and is not very immunogenic. Recently, monoclonal antibodies specific for CAT have been obtained using partially purified preparations of bovine and rat CAT (Levey et al 1983, Crawford et al 1982, Eckenstein & Thoenen 1982). These antibodies immunoprecipitate CAT, bind to at least three non-overlapping sites on the enzyme, and bind cholinergic neurons in sections of brain. Although some of the antibodies are species-specific, others bind CAT in a wide variety of species. These antibodies provide the first unambiguous markers for cholinergic neurons.

Other Antibodies that Define Neuronal Subclasses

The most successful strategy for isolating monoclonal antibodies that bind subclasses of neurons has been to immunize mice with material from one part of the nervous system and screen the antibodies secreted by the hybridomas on sections or cultures. Antibodies specific for peripheral and central neurons
have been identified (Vulliamy et al 1981, Cohen & Selvendran 1981). Antibodies to retinal membranes have been identified that distinguish photoreceptors, other neurons, and Muller cells from each other (Barnstable 1980, Barnstable et al 1983, Trisler et al 1983). Antibodies to other regions of the mammalian CNS distinguish many of the different neurons within these regions. Striking among these are a series of antibodies, originally isolated using *Drosophila* nervous tissue as an immunogen, that cross-react with the human nervous system (Miller & Benzer 1983). Some of these antibodies stain subpopulations of neurons and others stain particular areas within individual neurons. When analyzed in immunoblots, the *Drosophila* and human antigens often are closely similar, suggesting an extraordinary conservation of antigenic epitopes (see also Ventner et al 1984). Another striking antibody, CAT 301, isolated using cat spinal cord as an immunogen, binds in the cerebellum only to a surface or extracellular matrix protein associated with the cells of Lugaro, a very rare cell type (McKay & Hockfield 1982, Hockfield & McKay 1983a). This antibody also binds elsewhere in the CNS, including pyramidal cells in the hippocampus and a variety of cells in area 17 of the visual cortex. This antibody exhibits particularly intriguing binding specificity to cells in the visual system of monkeys and cats (Hendry et al 1984). In the monkey lateral geniculate nucleus, binding is primarily restricted to magnocellular, not parvocellular, layers. In area 17, patches of stained cells are seen in layers III, IVB, and VI. These patches line up radially with each other and with the centers of ocular dominance columns. The distribution of the CAT 301 antibody suggests that it distinguishes cell groups with particular functions in the lateral geniculate nucleus and visual cortex. Conceivably, injection of this antibody coupled to ricin or another cytotoxic agent could be used to kill these cell populations in order to examine in more detail their roles in processing visual information.

Specific glycolipids, defined by antibodies, have been found to be associated with subpopulations of neurons (Richardson et al 1982), most notably subsets of sensory neurons (Dodd et al 1984). As these antigens are resistant to proteases, they should be particularly useful for separating neurons.

### Neuron-specific Antigens in Invertebrates

Monoclonal antibodies to neural antigens in invertebrates, especially the leech and insects, have revealed a large degree of antigenic diversity among neurons (e.g. Zipser & McKay 1981). Many of the antibodies directed to antigens in the leech nervous system define small groups of cells, for example subsets of sensory neurons and motoneurons. The antibodies distinguish a very large number of different sets of neurons, some of which are nested within others (Hogg et al 1983). The antibodies have made it possible to identify all the neurons of a particular type in the leech nervous system and have revealed...
relationships between neurons that were not previously recognized (Zipser 1982, Zipser et al 1983). Examination of the position of axons stained by individual antibodies has shown that they have consistent positions in connectives (Hockfield & McKay 1983b). In some cases, antigens mark axons clustered into fascicles that are derived from scattered cell somata; in other cases, antigens mark clusters of cell somata whose axons are dispersed into different fascicles. Thus, the factors important for aggregating cell somata and nerve bundles must be different.

Monoclonal antibodies directed against Drosophila and grasshopper neural tissues also reveal a large diversity in the antigenic properties of different neurons (e.g. Fujita et al 1982, Aceves-Pina et al 1982, Kotrla & Goodman 1982). Antigens specific for the neurophil, cortex, and nerve fibers are readily distinguished (Fujita et al 1982). Focusing on the compound eye, Benzer & colleagues (Fujita et al 1982) isolated antibodies that labelled the lens, the underlying crystalline cone, the secretory cone cells, and the photoreceptors. Several of these were used to study the development of the compound eye (Zipursky et al 1984). The $M_r$'s of many of these antigens have been successfully estimated by immunoblot analysis.

**MONOCLONAL ANTIBODIES THAT PROBE DEVELOPMENT OF THE NERVOUS SYSTEM**

**Determination of Neural Crest Cells**

Multiple cell types, including neurons and Schwann cells in the peripheral nervous system, are derived from the neural crest (reviewed in Le Douarin et al 1981). Transplantation experiments have shown that populations of crest cells can differentiate differently depending on the environmental stimuli that they encounter. An important issue, now being addressed using monoclonal antibodies, is whether cells in the crest are truly pluripotent or whether subpopulations with restricted fates are generated before crest cell migration. A monoclonal antibody, termed NC-1, that binds to a cell surface antigen on avian neural crest cells has recently been isolated and used to examine neural crest cell migration (Vincent et al 1983). With this antibody, it is possible to examine neural crest cell migration in normal animals. Previously, this required experimental manipulation, usually transplantation. Non-neural derivatives of the neural crest, such as melanocytes and mesenchymal cells in the branchial arches, do not bind the NC-1 antibody, but do appear to be derived from NC-1--positive cells (Vincent & Thiery 1984), a result consistent with a pluripotent precursor population of crest cells. A complementary monoclonal antibody, E/C8, that binds mesenchymal cells in the branchial arches, but not neural crest cells, has been isolated by immunizing with avian sensory neurons (Ciment & Weston 1982). It has been possible to show that E/C8-positive mesen-
chymal cells from the third and fourth branchial arches can develop into neurons, but not melanocytes in vitro (Ciment & Weston 1983). They can invade aneural gut to form enteric ganglionic neurons in organ culture, but are not competent to form melanocytes in developing embryos. It should be possible to determine whether loss of the NC-1 antigen or acquisition of the E/C8 antigen is related to the loss by cells in the branchial arches of competence to differentiate into melanocytes. The E/C8 antibody also binds neuronal cells in both the central and peripheral nervous systems. It binds to neuronal precursors at an earlier stage than any other known neuronal marker. For example, it appears in the future sites of sensory ganglia a day before condensation of these ganglia. It should consequently be very useful in studying early steps in neuronal commitment and differentiation.

Although the previous results suggest that neural crest cells are homogeneous before migration, monoclonal antibodies specific for ciliary neurons have been isolated that reveal heterogeneity in early neural crest cell cultures (Barald 1982). Two antibodies bind a small percentage of cultured neural crest cells provided the cells come from the region of crest that gives rise to the ciliary ganglion. Two different antigenic determinants have been defined by these antibodies, increasing the probability that they are related to cell lineage. This suggests that heterogeneity arises among crest cells at an early stage and results in the appearance of cell types that are partially restricted in their developmental potential. Positive and negative selections using antibodies to surface antigens that define neural crest subpopulations should be helpful for exploring crest cell lineages.

**Neuronal Cell Adhesion Molecules (N-CAM and Ng-CAM)**

Both Ca\(^{2+}\)-dependent and Ca\(^{2+}\)-independent adhesion systems have been detected in embryonic chick neural tissues (Brackenbury et al 1981). N-CAM, a glycoprotein that mediates Ca\(^{2+}\)-independent adhesion between chick neural cells, has been purified as a molecule that neutralizes antibodies, prepared against neuronal membranes, that inhibit neural cell adhesion (see Edelman 1983). Conventional antibodies to this glycoprotein were used to demonstrate the presence of N-CAM on the surface of the somata and processes of neurons, but not on other cell types in the chick brain. The antibodies prevented the formation of axonal bundles in sensory neuron cultures and disrupted histogenesis of developing retina in organ culture (Rutishauser et al 1978, Buskirk et al 1980). Very recently, the injection of N-CAM antibodies into the developing retina has been shown to disrupt the topological organization of retinal ganglion cell axons in the optic nerve and to prevent some of these axons from contacting their normal target in the optic tectum (Thanos et al 1984). These results argue that N-CAM is important in mediating interactions among neural cells in development. Monoclonal antibodies to N-CAM have recently been
isolated (Rutishauser et al 1982). Using these antibodies it has become possible
to purify milligram quantities of N-CAM for biochemical investigations,
including binding studies (Rutishauser et al 1982), and chemical characteri-
zation (Hoffman et al 1982). Using affinity-purified N-CAM, it has been
possible to detect differences in the carbohydrate attached to embryonic and
adult brain N-CAM molecules and show that these differences alter the adhe-
Monoclonal antibodies with cross-species reactivity have also been used to
detect and purify a very similar N-CAM from rodent brain (Chuong et al
1982), and to show that mice homozygous for the neurological mutation stag-
gerer do not synthesize the adult form of N-CAM in the cerebellum, the site
at which this mutation affects histogenesis (Edelman & Chuong 1982).

More recently, similar strategies using monoclonal antibodies have been
used to purify and characterize other molecules that mediate cell adhesion. A
partially purified tryptic fragment of a molecule that mediates neuronal adhe-
sion to glia has been used to raise monoclonal antibodies that inhibit the
binding of neuronal membranes to glia (Grumet et al 1984). The monoclonal
antibodies have been used to purify the intact, unproteolyzed form of this
glycoprotein, termed Ng-CAM, which differs from N-CAM in having a $M_r$
of 135,000 and a different pattern of peptide fragments generated by pro-
teolysis. Ng-CAM can be detected by immunofluorescence on neurons, but
not on glia. It is found on the same neurons as N-CAM. Despite the chemical
differences between N-CAM and Ng-CAM, some monoclonal antibodies bind
both glycoproteins, and the conserved determinants do not appear to be on
carbohydrate: This suggests that there is a shared protein domain on these two
different cell adhesion molecules. A trypsin fragment of a cell adhesion mol-
ecule, liver-CAM (L-CAM), has been purified by the same strategy with
monoclonal antibodies (Gallin et al 1983). This $Ca^{2+}$-dependent adhesion
molecule has a wide but specific distribution in both the embryo and adult
(Edelman et al 1983, Thiery et al 1984). It appears to be lost from cells when
they become committed to neuronal differentiation.

**Neuronal Cell Migration**

Studies with conventional antibodies have demonstrated strong spatial and
temporal correlations between the appearance of fibronectin and the migration
of granule cells to form the external granule cell layer in the cerebellum (Hatten
et al 1982) and migration of neural crest cells to form the variety of tissues
derived from the crest (Thiery et al 1982a,b). When cerebellar granule cells
or neural crest cells are cultured in vitro, their abilities to adhere to and migrate
on fibronectin substrata correlate temporally with their migratory behaviors in
vivo (Hatten et al 1982, Rovasio et al 1983). In addition, N-CAM appears at
an appropriate time in vivo to be important in condensation of the sympathetic
and sensory ganglia from migrating crest cells (Thiery et al 1982b). Migration of cerebellar granule cells from the outer to inner granule cell layer can occur in vitro in brain slices. In a promising start in identifying molecules important in this migration, Schachner and colleagues have shown that mono- and polyclonal antibodies to L1, a neuronal cell surface glycoprotein, reduce the movement of granule cells in cultured slices of developing cerebellum (Lindner et al 1983). The polyclonal, but not monoclonal, antibodies to L1 also block a Ca\(^{2+}\)-independent aggregation of granule cells in vitro (Rathjen & Schachner 1984). L1 antisera bind to antigens of three distinct Mr, each of which corresponds in size to an antigen detected with antisera to Ng-CAM. If these two molecules are in fact the same, Ng-CAM is likely to guide granule cell migration.

**Axon Growth and Pathfinding**

A remarkable monoclonal antibody, 15, has been used in a series of experiments on the grasshopper nervous system to study the mechanisms by which axons grow along stereotypic pathways to reach their targets. This antibody stains a specific subset of neurons, including peripheral pioneer neurons (Ho & Goodman 1982). Since it stains all parts of these neurons, even before the initiation of axon growth, it has been possible to use this antibody to study the pathways and contacts made by pioneer fiber growth cones. The 15 antibody stains an array of previously unseen pioneer neurons that lay down different segments of the early fiber tracts. Identification of these cells has made it clear that pioneer neurons and other landmark cells are quite close to each other during initial fiber growth—within the reach of filopodia in neighboring growth cones. Consequently, pathways may be constructed by sequential movement of growth cones from one of these landmark cells to the next. Evidence for preferential filopodial adhesion to landmark cells has been obtained by comparing at the electron microscope level the number of filopodia adhering to landmark vs other cells, using Lucifer Yellow to fill the filopodia of individual neurons and an antibody to Lucifer Yellow to visualize the filled processes (Taghert et al 1982). The 15 monoclonal antibody also stains a previously unrecognized class of mesodermal cells that arise early in development (Ho et al 1983). Their pattern of growth and development, revealed by staining with the 15 antibody, indicates that they form a scaffold that guides the later development of tendons, muscles, and nerves. The nature of the antigen defined by the 15 antibody is not yet known, but it is clearly associated with a set of cells with important roles in development.

Studies in both vertebrates and invertebrates have shown that the growth cones of different neurons will make divergent choices at branch points (e.g. Raper et al 1983a,b). A monoclonal antibody has recently been isolated that inhibits axon growth by avian retinal ganglion cells, but not sensory neurons (Henke-Fahle & Bonhoeffer 1983). The antigen defined by this antiserum
could be one member of the family of molecules that guide these disparate decisions.

Trophic Factors

Many factors have been described that regulate the expression of differentiated functions in neurons. These include substances essential for survival, proliferation, transmitter choice, neurite growth, and steps in synapse formation. Immunological studies will clearly be critical in establishing their biological relevance.

Nerve growth factor (NGF) is a protein that is essential for normal development of both sensory and sympathetic neurons. It is the one neurotrophic factor, of many believed to exist, that has been purified, characterized, and demonstrated to have a role in normal development in vivo. The presence of NGF antibodies during development results in massive neuronal death in the sensory ganglia (Gorin & Johnson 1979). Injection of NGF antibodies at any time destroys the sympathetic nervous system (Levi-Montalcini & Booker 1960). Although mouse NGF acts on fish and avian neurons, antisera to NGF show strong species specificity (e.g. Harper et al 1983). Recently, several groups have isolated monoclonal antibodies to NGF and most of these prevent NGF binding to its receptor and NGF-dependent neurite outgrowth in vitro (e.g. Warren et al 1980, Zimmermann et al 1981, H. Thoenen, personal communication). The monoclonal antibodies also appear to show strong species specificity (H. Thoenen, personal communication). Recently, these antibodies have been used in two-step, sandwich-type antigen assays to measure for the first time the endogenous levels of NGF in tissues innervated by sympathetic neurons (Korsching & Thoenen 1983a). The same assay has been used to show that endogenous NGF is transported retrogradely from targets and is accumulated in sympathetic ganglia and on the distal side of nerve ligatures (Korsching & Thoenen 1983a,b). Since NGF is present at only a few nanograms per grams of tissue, it is very difficult to demonstrate that the antigen present in the tissue is NGF and not a cross-reacting antigen. Different monoclonal antibodies to NGF give similar results. If these antibodies can be shown to bind different epitopes on NGF, it will strengthen the argument that NGF and not a cross-reacting antigen is being detected.

Glial growth factor (GGF), a mitogen for Schwann cells and astrocytes, was found in pituitary and brain extracts (Brockes et al 1980). Although it was initially purified over 4000-fold by biochemical fractionation, the yield was very low. Monoclonal antibodies to GGF, prepared using a partially purified factor as an immunogen, have been invaluable for isolating larger quantities of the factor (Brockes et al 1981) and should facilitate future studies on its distribution and function. The monoclonal antibodies do not directly block the mitogenic activity of GGF, either because they do not bind a site essential
for activity or because their affinity is lower than that of GGF receptors. To remove activity, GGF must be precipitated with a second antibody.

**Markers of Cell Position**

Topological arrangements are preserved in the pattern of synapses formed between groups of neurons in many parts of the nervous system and are important for conveying spatial information. When axons from ganglion cells in the retina form synapses in the tectum, a point-to-point representation of the visual field is conserved. Sperry (1963) postulated that selective chemoaffinity generated by two orthogonal gradients of molecules provided the molecular basis for preserving this spatial order. In an effort to test this theory, Trisler et al (1981) have searched for antibodies that define cell position in the retina. A cell surface antigen, termed TOP, was identified that is distributed in a bilaterally symmetric dorsal-ventral gradient in the retina. At least 30-fold more antigen was found in dorsal than in ventral retina. The antigen was found on all cell types in fresh retinal dissociates. It appears in the optic cups of 2 day-old chick embryos, and the gradient is established before day 4. Thus a gradient is established early in retinal development, during the period of neuroblast proliferation, and is maintained after neurogenesis is completed. TOP has now been purified by affinity chromatography and shown to be a $M_r$ 47,000 cell surface glycoprotein (Trisler et al 1983). It remains to be determined whether the molecule has an important role in generating spatial order in the connections made by retinal ganglion cells with other parts of the brain.

A monoclonal antibody that recognizes a surface antigen whose expression is position-dependent has also been identified by using a grasshopper membrane fraction as an immunogen (Kotrla & Goodman 1982). The antibody, termed epi-1, stains an antigen on a broad strip of ectodermal epithelial cells near the midline in the blastula stage and a narrower strip of the same cells in the grasshopper gastrula. In later development, the antigen is restricted to two of the seven rows of neuroepithelial cells in each segment. The antigen is also positionally expressed on strips of cells near the distal end of each limb bud. In summary, screens for antibodies defining antigens that mark cell position have produced dramatic results in both vertebrate and invertebrate species and suggest that monoclonal antibodies to such molecules will be very useful in developmental studies.

**STRATEGIES FOR MAKING MONOCLONAL ANTIBODIES**

In this section, we review aspects of particular interest to neuroscientists regarding the methods for producing, screening, and productively using hybridomas and monoclonal antibodies. Numerous books and reviews on monoclonal antibod-
ies have been published in the last few years (e.g. Kennett et al 1980, Hurrell 1982).

**Isolation of Specific Hybridoma Cell Lines**

Detailed methods for producing monoclonal antibodies have been published elsewhere (e.g. Fazekas de St. Groth & Scheidegger 1980, Stahli et al 1980). Differences in procedures can result in selections of antibodies with different allotypes and specificities. It has therefore proven well worth the effort to prepare the immunogen, choose the immunization protocol, and select a screening procedure to optimize the probability of obtaining antibodies of the greatest interest and utility for future studies.

Recent work has shown that the probability of obtaining hybridoma cell lines is proportional to the strength of the immune response to a particular antigen (Lake et al 1979). Therefore, the first requirement for obtaining specific monoclonal antibodies is to induce a strong response to the desired antigen. Since inbred mouse strains differ significantly in their responsiveness to defined antigens, screening a variety of strains has proven useful when testing for an immune response. The original immunization protocols were optimized for injections of cells and membranes, and typically it was found that an optimal time for fusion of spleen and myeloma cells was three to four days after a final i.v. injection (Lake et al 1979). It now appears that hybridomas specific for soluble antigens are produced more efficiently by daily i.v. injections for three to four days prior to fusion, probably because soluble antigens are rapidly removed from the circulation (Stahli et al 1980).

Different allotypes are preferable for different purposes. IgM antibodies are excellent cytotoxic and agglutinating reagents, but are not as useful as IgG antibodies for many other purposes, including immunocytochemistry, which is crucial for many applications in neuroscience. Specific protocols for producing a high frequency of hybridoma antibodies of a specific allotype have been published (e.g. Colwell et al 1982). Immunization protocols of sufficient length to generate a high frequency of IgG-secreting hybridomas are usually preferable to short immunization regimens. Allotype-specific reagents can also be used in an initial screening to select for antibodies likely to be useful in future applications.

A major goal of neuroscientists has been to obtain cell-type-specific antibodies. The most successful fusions seeking these reagents have used fixed neural tissues as immunogens and have screened fixed tissue sections (Zipser & McKay 1981, McKay & Hockfield 1982). This strategy (a) minimizes the diffusion of antigens that can occur in unfixed tissue sections, (b) optimizes the morphology of cells, which may be unrecognizable in unfixed tissues, and (c) increases the probability that antibodies can be used in ultrastructural investigations. Since fixation can generate epitopes not present on unfixed
antigens, not all antibodies generated in this way will be useful for binding unfixed antigens, either on living cells or in immunoblots.

Interpretation of antibody binding patterns is almost always strengthened by identifying the molecules bound in each tissue by a particular monoclonal antibody. The most common procedures for doing this assay involve antibody binding to denatured antigens separated by size on polyacrylamide gels (e.g. Towbin et al 1979). To increase the probability of antibodies recognizing such antigens, denatured antigens have been injected in one step of some immunization protocols (Zipser & McKay 1981, McKay & Hockfield 1982, Flaster et al 1983).

Antibodies that cross species efficiently are naturally more widely applicable than those that bind only the species from which a particular antigen was derived. To increase the probability of obtaining such antibodies, neural material from more than one species has been used during the sequence of immunizations (e.g. Aceves-Pina et al 1982).

Another problem has been to identify monoclonal antibodies to minor epitopes in an antigen mixture. Monoclonal antibodies to the dominant antigens have been coupled to a resin and used to deplete an antigen mixture of previously identified antigens (Springer 1981). In another approach, cyclophosphamide, an alkylating agent known to kill antigen-stimulated lymphocytes preferentially, has been injected into previously immunized mice in an effort to kill lymphocytes specific for dominant antigenic determinants. Minor antigens have been recognized subsequently in surviving splenic lymphocytes that were challenged in vitro and fused to produce hybridomas (Matthew & Patterson 1983).

An often challenging problem has been to obtain a strong immune response to a minute amount of a neuronal antigen or to a functionally important and conserved part of such a molecule to which the mouse may be effectively tolerant. Microsequencing of natural proteins and chemical synthesis of milligram quantities of peptide sequences in these proteins has been one successful approach to overcoming such problems (Hunkapiller & Hood 1983, Sutcliffe et al 1983a,b). Immunization protocols in which lymphocytes are challenged with antigens in vitro provide another promising approach because they often require dramatically less antigen (picogram to nanogram) and can yield hybridoma lines derived from B lymphocytes that would be suppressed in vivo (e.g. Luben & Mohler 1980, Reading 1982, Pardue et al 1983). By including γ-interferon, it may prove possible in the future to obtain hybridoma clones in vivo and in vitro using even lower amounts of antigens (Nakamura et al 1984). In vitro protocols also have potential problems. The in vitro responses are often primitive compared to those in animals and a high proportion of the antibodies are IgM's. Too much antigen can easily result in isolation of hybridomas secreting low affinity antibodies. Prolonged in vitro
culture of lymphocytes with repeated challenges with low doses of antigen has been reported to produce a higher frequency (80%) of IgG-allotype-secreting hybridoma lines (Pardue et al 1983).

**Identification of Specific Hybridomas**

The most common screening procedures for monoclonal antibodies use enzyme-linked immunosorbent assays (ELISA) in which antibody binding to antigens adsorbed to solid supports is measured. These procedures can be modified to detect fewer than 100,000 molecules of bound IgG, a very high level of sensitivity (Shalev et al 1980). Many ELISA assays, though, require as much as a microgram of bound antigen, because much of the antigen is not accessible to antibody (Kennel 1982). As a result, whereas modern immunization procedures have made it possible to obtain hybridoma-secreting antibodies to sub-nanogram quantities of an antigen, it may be impractical to assay for antibodies binding such antigens in standard ELISA assays. NGF, receptors, and ion-selective channels, present typically in nanomolar concentrations, are examples of molecules that are too rare in most crude preparations to be detected in routine ELISA assays.

One way of overcoming this problem has been to precede hybridoma selection with a partial or complete purification of the molecule of interest. Antibodies to Nerve Growth Factor, the acetylcholine receptor, beta-adrenergic receptor, sodium channel, and choline acetyltransferase have been isolated in this way (Zimmermann et al 1981, Tzartos & Lindstrom 1980, Strader et al 1983, Moore et al 1982, Crawford et al 1982). A second approach has been to use substrates that bind antigens more efficiently and in more accessible forms than PVC plastic. Application of neuronal antigen mixtures to nitrocellulose provides an assay that requires about tenfold less antigen than standard solid phase assays (Hawkes et al 1982). Assays using a beta-galactosidase-immunoglobulin conjugate and a fluorogenic substrate have increased the sensitivity of assays for NGF and several other proteins approximately 100-fold (e.g. Korsching & Thoenen 1983a).

In many cases, solid phase immune assays have been replaced with a more specific assay. The most specific assays have involved tests of function or enzymatic activity. Antibodies to choline acetyltransferase and glial growth factor were identified by their abilities to precipitate each molecule (Crawford et al 1982, Brockes et al 1980). Monoclonal antibodies to the Na\(^+\) channel, muscarinic acetylcholine receptor, and \(\alpha_1\)-adrenergic receptor were identified by their abilities to precipitate \[^3H\]-saxitoxin, \[^3H\]-propylbenzilcholine mustard, and \[^3H\]-phenoxybenzamine, respectively, from partially purified preparations (Moore et al 1982, Ventner et al 1984). These screens are most successful when dealing with a purified antigen.

The most sensitive assays for cell-type specific monoclonal antibodies have
utilized histological screens of sections of nervous tissues (e.g. Zipser & McKay 1981, Hockfield & McKay 1981). In adequately preserved tissues, antibodies that bind specific subsets of cells and specific organelles can be recognized in sections in the light microscope. The most successful screens in sections of mammalian nervous systems have used frozen or Vibratome sections of fixed tissues (McKay & Hockfield 1982). Although time consuming, these screens are the only means of recognizing antibodies directed against specific subsets of neurons. Discussions of immunocytochemical procedures can be found in several reviews and are beyond the scope of this article (Sternberger 1979, Jones & Hartman 1978, Vaughn et al 1981).

SUMMARY

The preceding discussion documents the diverse ways in which monoclonal antibodies have contributed to neuroscience research. They provide highly specific reagents to membrane-associated proteins, such as pumps, channels, receptors, and cell-adhesion molecules, that are useful for purifying these proteins, studying their structures at high resolution, and mapping their distributions. In many cases, the specific reagents were obtained using only partially purified antigens. Monoclonal antibodies to cytoskeletal proteins, organelles, and protein kinases have revealed that specific molecules are concentrated in anatomically distinct regions of the cell. A protein kinase has been shown to be a major postsynaptic constituent in many synapses. Individual proteins, such as actin, tubulin, and calmodulin appear to have different antigenic epitopes shielded in different parts of the cell.

Monoclonal antibodies have provided a diversity of cell-type-specific reagents in both vertebrate and invertebrate nervous systems. They seem likely to be useful in identifying functionally related subpopulations of neurons and describing neural cell lineages. They will also serve to identify molecules that are important in regulating cell migration in the cerebellum, in marking cell position in the retina, and directing axon growth. This review also documents many purposes for which monoclonal antibodies are poorly suited or must be used with caution:

1. A monoclonal antibody to a protein does not always reveal every place where that molecule is located. Pre- or post-translational microheterogeneity can expose different epitopes on the protein, such as may occur on the Na\(^+\)-channel. Other proteins within the cell may shield antigenic sites on proteins such as calmodulin.

2. Monoclonal antibodies can bind to epitopes on unrelated molecules (Nigg et al 1982, Lane & Koprowski 1982). This is revealed in some cases as multiple bands on immunoblots. Some cross-reactivity, however, may have
a functional basis. For example, structural homology is clearly the basis for the antigenic epitopes that are shared among the five classes of intermediate filaments (Pruss et al. 1981). The epitope that appears to be shared between the muscarinic and α₁-adrenergic receptors may be conserved because the two receptors modulate common effectors. The cross-reactivity between these receptors was only recognized because very specific and sensitive assays exist for each. It is quite possible that these same antibodies also bind sites on many other types of receptors. Mapping the distribution of this epitope may therefore have little relationship to the actual distribution of the muscarinic receptor. (Antibody binding to proteins in polyacrylamide gels is probably not sensitive enough to detect these additional receptors.) If two antibodies to different epitopes on the same protein show the same pattern of binding, it greatly strengthens the interpretation of histochemical data. Antibodies to different peptides in the same protein have been used for this type of analysis (Sutcliffe et al. 1983a, b). With this same objective, antigens identified by monoclonal antibodies on polyacrylamide gels have been excised and used to generate additional monoclonal antibodies with the same binding specificity (Flaster et al. 1983).

3. Many molecules in the nervous system can be anticipated to be detected by immunocytochemistry, but not by other currently available procedures, so it may not be possible to identify the antigens defined by many monoclonal antibodies. Many other molecules of importance, such as the Ca²⁺-dependent K⁺ channel and NGF, exist at such low concentrations that the best current immunocytochemical procedures do not have the specificity and sensitivity needed to discern their distributions in the nervous system.

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