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106. Supported in part by NIMH grant MH 19691 and NIA grant AG 00538.

Immunological Approaches to the Nervous System

Louis F. Reichardt

In the past decade, immunological approaches have helped to revolutionize neurobiology. Monoclonal antibodies in particular have been versatile reagents and have provided a major impetus to research. In this review, we discuss examples of applications of antibodies to research in neuroanatomy, developmental neurobiology, neuronal cell biology, and protein structure. Antibodies have been used to map the distribution of molecules, both within the cell and throughout the nervous system. Often the use of antibodies has permitted the distribution of cells containing an antigen to be determined. Sometimes, this has led to the discovery of previously unappreciated relationships or differences among neurons. Antibodies have also helped to purify and characterize neuronal antigens, many of which were first identified with a monoclonal antibody. In some cases, related families of molecules have been discovered. Often antibody-blocking experiments have suggested functions for new antigens. Finally, "differentiation antigens," frequently defined by monoclonal antibodies, have been used to study many developmental problems.

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Nervous System Anatomy

Antibodies specific for major cell types. An important objective of neuroimmunology has been to develop reagents able to distinguish different subsets of cells in the nervous system. Antibodies to cell-surface antigens are particularly important for isolation of a cell type by positive or negative selection [reviewed in (*1*)]. Positive selection procedures include adhesion to antibody-conjugated resins or magnetic beads and cell sorting (*2*). Negative selection procedures include antibody-directed complement- or toxin-mediated killing of unwanted cell types (*3*).

The major cell types in the nervous system can now be distinguished with antisera to defined antigens [reviewed in (*4*)]. In the peripheral nervous system, for example, neurons, Schwann cells, and fibroblasts can be recognized by the use of antisera to tetanus toxin, laminin, and fibronectin (*5*). In the central nervous system, antisera to tetanus toxin, galactocerebroside, glial fibrillary acidic protein, and thy-1 serve to distinguish neurons, oligodendrocytes, astrocytes, and ependymal cells (*6*). Monoclonal antibodies specific for many of these cell types have also been isolated [reviewed in (*4*)].

Antibodies specific for neuropeptides and transmitters. The most straightforward way to detect differences between neurons has been to use antibodies specific for different transmitters. During the past decade, immunocytochemical methods have shown that more than 30 peptides, often previously identified elsewhere, are localized in specific neurons, coexisting in many areas with other peptides or classical transmitters (*7*). Techniques in molecular biology have revealed even larger numbers of peptides encoded in the genes for the precursors to these peptides. Tissue-specific RNA splicing and prohormone processing have further expanded the numbers of known peptides (*8*). Since only a few of the genes encoding these peptides have been examined, there is scope for further growth in the number of identified peptides. Antibodies to synthetic peptides containing specific epitopes, especially those that can distinguish between related peptides, should be particularly valuable for future work. In some cases, behavioral responses have been shown to require more than one of the peptides encoded by a single gene (*9*).

The use of antibodies has not been restricted to peptide transmitters. It has been possible to visualize amine transmitters, including serotonin, glutamic acid, and γ-aminobutyric acid (GABA), by the use of antibodies to these amines coupled to protein carriers (*10, 11*). This approach should be particularly useful in identifying neurons utilizing amino acids as transmitters—for example, glutamic acid and aspartic acid. Antibodies to enzymes required for the synthesis of other transmitters, such as GABA, catecholamines, and acetylcholine (ACh) have been used for many years to identify neurons likely to contain these substances. In recent years, monoclonal antibodies specific for several of these enzymes, including glutamic acid decarboxylase, tyrosine hydroxylase, dopa-

mine-β-hydroxylase, and choline acetyltransferase (CAT), have been isolated (*12*). These have been particularly useful in studies on the latter enzyme, which is difficult to purify and which is only weakly immunogenic. Recent studies with antibodies to transmitter enzymes have suggested that classical transmitters coexist in many cell types (*13*).

A few applications of monoclonal antibodies merit special mention. Monoclonal antibodies to substance P and serotonin have been internally labeled by growth of the hybridoma cells in [^{3}H]lysine. Fab fragments, whose small size improves penetration, can then be used to determine the locus of each transmitter by high-resolution autoradiography (*10*). Cell lines secreting antibodies in which one antigen-binding site binds a neurotransmitter and the other binds horseradish peroxidase have been derived from fusion of transmitter-specific and peroxidase-specific hybridomas (*14*).

Other antibodies specific for neuronal subclasses. The most successful strategy for identifying monoclonal antibodies specific for subclasses of neurons has been to screen the antibodies on sections of neural tissue. The most striking results have been obtained with invertebrate neural tissues, most notably from grasshopper, *Drosophila melanogaster*, and leech. For example, antibodies that label several elements of the compound eye in *Drosophila*, including the lens, underlying crystalline cone, secretory cone cells, and photoreceptors, have been identified and used to study the development of that sensory organ (*15*). As another example, many of the antibodies isolated with the use of the leech nervous system have been shown to define small groups of neurons that are often related functionally—for example, subsets of sensory neurons (*16*).

It is also possible to isolate antibodies specific for subsets of vertebrate neurons (*17*). Striking among these are a series of antibodies, originally isolated by immunizing with *Drosophila* nervous tissue, that cross-react with the human nervous system (*18*). In many cases, the sizes of the proteins recognized by a particular antibody are similar in *Drosophila* and humans, suggesting a surprising degree of antigenic conservation. Another valuable antibody, CAT 301, isolated after immunization with cat spinal cord, binds in area 17 of the visual cortex to patches of cells in layers III, IV B, and VI, which line up radially with each other, mark the centers of ocular dominance columns, and may well be related functionally (*19*).

Glycolipids associated with specific neuronal subpopulations have been identified with antibodies and should be particularly useful for cell separations (*20*). Cell separation procedures can also be used in ways independent of surface antigens. The fluorescence-activated cell sorter (FACS) makes it possible to separate neurons that innervate particular regions, because these neurons will internalize and retrogradely transport fluorescent ligands injected into the vicinity of their axons' terminals. Motoneurons have been purified as fluorescent cells in spinal cord dissociates after injection of a fluorescent lectin into embryonic chick muscles (*21*). More specific neuronal populations can be labeled by injecting fluorescent microspheres which remain within a few microns of an injection site (*22*).

Cell position markers. Topological relationships are important in development and function of the nervous system, and screens for monoclonal antibodies that mark cells according to their position have produced dramatic results. One particular antigen, termed TOP, is a 47-kilodalton (kD) cell-surface glycoprotein that is distributed in a dorsal-ventral gradient in the avian retina (*23*). There appears to be at least a 30-fold difference in concentration of TOP between the dorsal and ventral aspects of the retina. The gradient is established early in retinal development, during the period of neuroblast proliferation. The antigen is found on all cell types in freshly dissociated cultures. It has been proposed that selective chemoaffinity, generated by two orthogonal gradients of molecules, provides a molecular basis for conservation of a point-to-point representation of the visual field when axons from retinal ganglion cells innervate the tectum (*24*). It will be interesting to determine whether injection of TOP antibodies disrupts this representation in the tectum.

Summary. Immunology has had a major impact on neurobiology, expanding dramatically the number of subjects amenable to investigation. Studies with antibodies to neuropeptides, transmitters, and transmitter enzymes have disclosed a great heterogeneity among neurons and have provided clues for interpreting anatomical connections. Monoclonal antibodies are being used to identify functionally related subpopulations of neurons and cell lineages in development and to study mechanisms by which axons grow along stereotypic pathways to reach their targets. Other antibodies have identified molecules that appear to participate in cell aggregation, cell migration, cell position, and axon growth. Antibodies have revealed that many proteins are concentrated in anatomically distinct regions of the neuron. Moreover, these studies have suggested that individual proteins have different antigenic epitopes shielded or modified in different parts of the same neuron. Antibodies to membrane proteins crucial for neuronal function, such as ion pumps, ion-selective channels, and receptors, have been used to map their distributions and to study their structures at high resolution.

Nervous System Development

Neuronal cell migration and aggregation. Components of the extracellular matrix and cell surface are believed to be important in determining the structure and properties of neurons. During the past decade, antibodies have made it possible to identify many important cell-surface molecules and are beginning to be used to study the roles of these molecules in directing development in vivo. Studies on the neuronal cell adhesion molecule (N-CAM) provide the most complete work illustrating the use of antibodies. N-CAM is a glycoprotein that mediates Ca^{2+}-independent adhesion between vertebrate neural cells (*25*). N-CAM was purified as a molecule that neutralized antibodies to neuronal membranes, which blocked neural cell adhesion (*26*). Once purified, specific antibodies were prepared and used to show that N-CAM was found on neurons in culture (*27*). Monoclonal antibodies have more recently been used to purify milligram quantities of N-CAM for binding studies and chemical characterization. It has been possible to show that N-CAM molecules derived from the embryonic and adult brains have different amounts of carbohydrate, and these differences alter the adhesive properties of N-CAM (*25*).

More recently, similar immunological assays have been used to purify two other molecules that mediate cell adhesion. One molecule, liver-CAM (L-CAM), is a Ca^{2+}-dependent adhesion molecule with a wide, but specific, distribution in both the embryo and adult (*25, 28*). It appears to be lost from cells when they become committed to neuronal differentiation. The other molecule, neural-glial CAM (Ng-CAM), appears to mediate the adhesion of neurons to glia (*27*). Ng-CAM can be detected by immunoflu-

orescence on the same neurons as N-CAM and appears not to be on glia. Despite differences in binding specificity between N- and Ng-CAM, some monoclonal antibodies bind to both molecules, suggesting some homology.

Antibodies have shown that each cell adhesion molecule appears at specific times and places, suggestive of important roles in development (*25, 28*). L-CAM, for example, first appears in the mouse morula. Antibodies to L-CAM inhibit the aggregation of isolated morula cells, prevent the initial compaction of the blastula, and prevent the appearance of a differentiated inner cell mass, possibly by disrupting the polarity of the division of blastomeres at the eight-cell stage of development (*29*). Polyclonal, but not monoclonal, antibodies to N-CAM disrupt histogenesis in embryonic retinas explanted into culture, prevent the formation of axonal bundles by sensory neurons, and reduce the affinity of neurons for cultured myotubes (*30*). Injection of N-CAM antibodies into developing chick retinas prevents some retinal ganglion cell axons from following normal pathways across the surface of the eye (*31*). These displaced axons also follow aberrant pathways in the optic nerve, and many fail to contact their normal targets in the optic tectum.

Polyclonal, but not monoclonal, antibodies to L1, a glycoprotein identified initially with a monoclonal antibody, block the aggregation of cerebellar granule cells in vitro (*32*). In vivo, cerebellar granule cells migrate from the outer to the inner granule cell layer in close apposition to radial glial fibers. Migration of these granule cells also can occur in vitro in brain slices and is reduced by mono- and polyclonal antibodies to L1 (*32*). Antigens of three distinct molecular sizes are bound by L1 antisera, and each corresponds in size to an antigen detected with antisera to Ng-CAM. It seems possible, therefore, that Ng-CAM is L1 and is required for granule cell migration along radial glia.

Axon growth and pathfinding. Insight into the mechanisms by which axons grow along stereotypic pathways during embryogenesis has been provided by antibodies to insect nervous systems. Antisera to horseradish peroxidase and a monoclonal antibody, I5, both stain the pioneer neurons responsible for establishing initial axon pathways (*33*). These antibodies have revealed an array of previously unseen pioneer neurons and landmark cells. By using the antibodies to follow the development of these cells it has become clear that different neurons establish different segments of the early fiber tracts, and these neurons are initially close enough to each other to be within reach of filopodia in neighboring growth cones. By using Lucifer yellow to fill these cells and an antibody to Lucifer yellow to visualize the filled processes in the electron microscope, it has been possible to show that filopodia adhere preferentially to landmark cells (*34*). These observations make it seem likely that the formation of axonal pathways is guided in large part by selective adhesion, mediated in part by the sequential movement of growth cones from one landmark cell to the next (*35, 36*). The I5 antibody also stains a previously unrecognized class of mesodermal cells that arise early in embryogenesis and appear to form scaffolds that direct the later formation of nerves, tendons, and muscles (*37*).

The growth cones of different neurons clearly make divergent choices at branch points and follow different pathways (*36*). Any of the surface antigens that distinguish neurons during embryogenesis are plausible candidates to mediate these decisions. A particularly promising candidate is the antigen defined by a monoclonal antibody that has recently been shown to inhibit axon growth by some, but not all, classes of avian neurons (*38*).

Cell determination and lineage. The neural crest (NC) gives rise to many cell types, and transplantation experiments have shown that the environment is important in regulating the differentiation of these cells (*39*). It is uncertain, however, whether early NC cells are truly pluripotent. A monoclonal antibody to a cell-surface marker for avian NC cells, termed NC-1, has recently been isolated and used to examine NC cell migration and pluripotency (*40*). Non-neural derivatives of the NC, such as melanocytes and mesenchymal cells in the branchial arches, do not bind the NC-1 antibody, although they appear to be derived from NC-1-positive crest cells. Another monoclonal antibody, E/C-8, isolated with avian sensory neurons as an immunogen, appears on crest-derived mesenchymal cells in the branchial arches (*41*). E/C-8–positive mesenchymal cells develop into neurons, but not melanocytes, in vitro. If transplanted, they will invade the gut to form neurons in organ culture but will not form melanocytes in vivo. It will be interesting to use these two antibodies, NC-1 and E/C-8, in combination to determine when competence to differentiate into melanocytes is lost. Although the previous results suggest that NC cells become heterogeneous only after initiating migration, some monoclonal antibodies have been isolated that stain subpopulations of early NC cells. Two monoclonal antibodies specific for ciliary neurons have been isolated, with these neurons used as an immunogen (*42*). They bind a small percentage of NC cells derived from the region that gives rise to the ciliary ganglion but do not bind crest cells from other regions. These antibodies should make it possible to determine when the ciliary lineage is established.

The markers to major cell types have also been used for studying many types of developmental questions, including cell lineage relationships and the requirements for synthesis of myelin (*43*).

Trophic factors. In the well-known experiment establishing the importance of nerve growth factor (NGF), injection of NGF antibodies into neonatal mice induced massive cell death in sympathetic ganglia (*44*). In embryos, such antibodies also destroyed much of the sensory nervous system (*45*). Despite these experiments demonstrating the importance of NGF and other experiments demonstrating that these neurons obtain an essential trophic factor from their targets, it has been difficult to show that NGF is actually present in sympathetic effector organs. Only recently, with a sensitive two-site enzyme immunoassay, has it been possible to detect endogenous NGF in sympathetic targets (*46*). The same assay has been used to show retrograde transport of endogenous NGF to the sympathetic and sensory ganglia. These experiments are a model for studies on the role of other trophic factors in development of the nervous system.

Extracellular matrix composition at the neuromuscular junction. The extracellular matrix (ECM) between the pre- and postsynaptic elements of the neuromuscular junction has become a subject of intense interest, because it is able to induce synaptic specializations, including aggregation of ACh receptors, in regenerating nerves and muscles (*47*). Several antibodies, some monoclonal, distinguish junctional and extrajunctional ECM (*48, 49*). Antibodies to ACh esterase, a proteoglycan, and a substantially purified factor that induces aggregation of ACh receptors in cultured myotubes bind specifically to synaptic regions of the ECM (*47, 50*). Antibodies to certain collagens, including collagen V, bind in a pattern indicating that these proteins are actually excluded from the synaptic ECM (*49*). It will be particularly interesting to determine whether anti-

bodies to the ACh receptor–aggregating activity, already shown to inhibit receptor aggregation in vitro, can also block steps in synapse formation in vivo.

Protein and Cell Structure

Acetylcholine receptor. The ACh receptor is a pentameric protein complex, consisting of four different, but homologous subunits, that form a transmembrane channel which is opened by the binding of ACh [reviewed in (*51*)]. As the primary target in a human autoimmune disease and an animal model—myasthenia gravis (MG) and experimental autoimmune myasthenia gravis (EAMG)—it has long been of interest to immunologists. Recent studies with monoclonal antibodies to the receptor and antibodies to synthetic peptides illustrate the potential for applying immunological methods to intensive studies on the structure and function of single proteins or protein complexes.

Using monoclonal antibodies, 28 different determinants on denatured *Torpedo* receptor subunits have been identified (*52*). These include determinants unique for individual subunits and determinants shared by more than one subunit (*53*). Antibodies to determinants shared by more than one subunit provided the first evidence for homologies between subunits, since confirmed by sequencing the genes encoding each subunit (*54*). Epitopes defined by monoclonal antibodies are being used to probe the structure of each receptor subunit and, in particular, to identify extracellular and cytoplasmic domains (*55*). The results are generally consistent with models of subunit structure derived from hydrophobicity of the amino acid sequence. An antibody to a synthetic peptide corresponding to the carboxyl terminus of the δ subunit has been shown to bind to the cytoplasmic side of the membrane (*56*), arguing persuasively that receptor subunits have an amphipathetic transmembrane alpha helix in addition to the four hydrophobic transmembrane helices identified by analysis of hydrophobicity. Fab fragments are also being used in high-resolution electron microscopy studies to map the positions of the subunits, which formed a pentameric rosette around a central channel (*57*).

Monoclonal antibodies to the ACh receptor have been used to examine the etiology of MG. The results of competitive binding assays in which the monoclonal antibodies are used to determine the specificities of the antibodies to the ACh receptor present in myasthenic sera provide evidence that MG patients produce antibodies to the same regions on the ACh receptor as animals immunized with the receptor; this indicates that antibody production in MG is stimulated by the ACh receptor, not by a cross-reacting antigen (*58*). The ability of different monoclonal antibodies to induce passive EAMG after injection in vivo has been shown to correlate with their ability to aggregate solubilized receptor in vitro (*59*). As expected, only antibodies to the α subunit, two copies of which are in each receptor monomer, are able to induce the formation of aggregates larger than dimers. Only those antibodies to the α subunit that promote formation of these larger aggregates can induce EAMG or increase turnover of ACh receptor.

Antibodies to other receptors. Monoclonal antibodies specific for several other receptors, including the β- and α_1-adrenergic, muscarinic ACh, epidermal growth factor, and NGF receptors, have been obtained by immunizing with either plasma membrane or purified receptor preparations (*60–62*). With these antibodies, the β-adrenergic receptor has been shown to be concentrated in postsynaptic densities (PSD's). Antibodies have been used to show that all identified antigenic epitopes on the muscarinic receptor are conserved in all tissues and species examined, including invertebrates. The muscarinic and α_1-adrenergic receptors have been shown to share antigenic sites, possibly because they modulate common effectors.

Antibodies to ion-selective channels and pumps. Ion-selective channels and pumps have been long recognized as being responsible for regulating the electrical properties of neurons and the membrane potential, which controls the conductance of Ca^{2+} channels, thereby serving as the most important regulator of transmitter release. During the past few years, both poly- and monoclonal antisera to the Na^+ channel have been prepared. This is the one channel that has been significantly purified (*63, 64*). Each antibody binds specifically the large (about 270 kD) glycoprotein subunit of the channel. A polyclonal antiserum to a highly, but not completely, purified *Electrophorus* Na^+ channel preparation has been shown to bind to the innervated surfaces of electrocytes and to nodal regions of myelinated axons, two regions rich in Na^+ channels (*63*). Intriguingly, a monoclonal antibody was found to bind only to the innervated faces of the electrocytes and not to nodes of Ranvier, suggesting a difference between the Na^+ channels on electrocytes and nerves (*65*). This antibody has proven particularly useful for completing the purification of this channel (*66*). More detailed studies with these antibodies, and antibodies to other channels, should improve our understanding of how the distribution of channels controls the electrical excitability of neurons.

The Na^+-K^+ exchange pump, which contains two protein subunits of approximate 120 and 50 kD is the major mechanism for removing internal Na^+ and restoring internal K^+ after action potentials. Antibodies to this pump have been used to determine its distribution in fish brain, where it appears to be concentrated in nodes of Ranvier and to be distributed over the surfaces of neuronal somata and dendrites (*67*). Two classes of nerve terminals, differing in their apparent concentrations of Na^+-K^+ pump, were observed in these experiments, and this difference could have important functional consequences. More recently, a monoclonal antibody to the chick Na^+-K^+ pump has been isolated (*68*). This antibody binds the Na^+-K^+ pump in only a subset of the avian cells known to contain this pump and binds to a nonglycosylated form of the pump, arguing for molecular heterogeneity in Na^+-K^+ pump polypeptides. More definitive analysis will undoubtedly be provided by gene cloning. In contrast to the earlier results with fish brain, this antibody detected high concentrations of the Na^+-K^+ pump in internodal regions of chick myelinated nerves. The reason for this apparent discrepancy is not clear.

Cytoplasmic Ca^{2+} levels, crucial for regulation of exocytosis, are controlled by several Ca^{2+} removal systems, including pumps and antiporters. Two major brain adenosine triphosphate (ATP)–dependent Ca^{2+} translocators have been purified, only one of which appears to be regulated by direct binding of Ca^{2+}-calmodulin (*69*). While the Ca^{2+}–calmodulin-binding transporter appears to be identical to the Ca^{2+} translocator in other tissues, monoclonal antibodies to the other transporter do not cross-react with major non-neuronal sources of Ca^{2+}-adenosinetriphosphatases, such as the sarcoplasmic reticulum and red blood cell. The ultrastructural localization of this putatively specific neuronal transporter will be of great interest.

Cytoskeletal elements. Neurons contain actin, tubulin, and intermediate filament networks which have been examined in detail with various antibodies. Antibodies specific for each subunit of

the neurofilament triplet stain the same filaments (*70, 71*). Antibodies to the 195-kD subunit bind to crossbridges, whereas antibodies to the two smaller subunits bind to the cores of the filaments. All three subunits appear only in neurons, but some neurons, such as cerebellar granule cells, do not bind antibodies to any subunit (*70, 72*). In some neurons, the large subunit—but not the other subunits—appears to be much more prominent in axons than in cell bodies or in dendrites (*71, 73*). Some of these apparent differences in distribution of the neurofilament large subunit may actually reflect differential phosphorylation of that subunit. Monoclonal antibodies specific for both phosphorylated and nonphosphorylated forms of the large subunit have been isolated (*74*). These antibodies stain preferentially different neurons and parts of neurons. The staining pattern suggests that the large subunit is much more heavily phosphorylated in axons than in dendrites of Purkinje cells.

A differential distribution of other cytoskeletal proteins has also been shown in Purkinje cells. With monoclonal antibodies, the microtubule-associated proteins, MAP_1 and MAP_2, have different distributions, with MAP_2 restricted to dendrites and MAP_1 much more broadly distributed (*75, 76*). Different monoclonals to actin and MAP_2 exhibit differential staining patterns in the Purkinje cell dendrite shaft and spine (*75*), suggesting that these two proteins are modified differently or interact with different molecules in these two compartments. Monoclonal antibodies specific for the tyrosylated- and nontyrosylated forms of α-tubulin stain the axons of cerebellar granule cells differently, depending on their age (*77*). This probably reflects reduced tubulin tyrosylation in more mature axons.

Antibodies to brain-specific and erythrocyte-specific spectrins have been used to show the presence of both forms in neurons, where the erythrocyte form appears at a terminal stage in differentiation and is restricted to the cell soma and dendrites (*78*). As spectrins bind to both the membrane and cytoskeleton, previously described differences in membrane and cytoskeletal composition may be related.

Organelles. A neuron-specific mitochondrial protein has been identified with one monoclonal antibody (*79*). Several proteins, including the phosphoprotein synapsin (*80*), have been shown with monoclonal antibodies to be associated with the membrane of synaptic vesicles. These antibodies have been used to identify antigens shared by many classes of vesicles, to purify vesicles, to monitor the cycling of vesicle membranes or contents, and to monitor nerve terminal differentiation in vivo and in vitro (*81*).

Although components of synaptic vesicles are the only antigens defined at present that are concentrated in the presynaptic nerve terminal, antibodies have made a much more significant contribution to characterizing the molecular constituents of postsynaptic specializations. Where receptor antibodies are available, such as to the β-adrenergic receptor, they have localized these receptors to PSD's in the central nervous system (*60*). The PSD's, which include a lattice of microtubules and microfilaments, have been stained with antibodies to many cytoskeletal proteins, including β-tubulin, actin, MAP_2, and brain spectrin (*75, 82*). Antibodies specific for calmodulin and for two Ca^{2+}-calmodulin–activated enzymes—a protein kinase and protein phosphatase 2B—have been used to show that these molecules are in many PSD's (*83*).

An analogous set of proteins, including the ACh receptor, has been localized with toxins and antibodies to the neuromuscular junction. Antibodies to a 43-kD protein that copurifies with the ACh receptor have recently been used to visualize the protein in the electron microscope as a dense bar of material associated with the cytoplasmic domains of the receptor (*84*) and to provide biochemical evidence for a specific association (*85*). A monoclonal antibody has been used to show that the 43-kD protein binds ATP and is a protein kinase (*86*). Proteins with probable structural roles that have been localized to the neuromuscular junction include actin, three actin-binding proteins (*alpha*-actinin, vinculin, and filamin), and an antigen cross-reactive to intermediate filaments (*87*).

Conclusion

The preceding sections illustrate a few of the ways in which antibodies have contributed to recent progress in neurobiology. Monoclonal antibodies, in particular, have provided many cell-specific reagents that seem likely to be useful in separating cells and addressing many problems in development. These problems include cell lineage relationships, the consequences of interaction between different cells, and identification of molecules important in regulating cell aggregation, migration, position, axon growth, and synapse formation. On the subcellular level, monoclonal antibodies have provided highly specific reagents for receptors, pumps, channels, organelles, cytoskeletal proteins, and protein kinases. They have already shown that some proteins are concentrated in anatomically distinct regions of the cell, while the modification of others depends on their subcellular position. Although the significance of such differences is mostly not known, the immunological data are making it possible to address questions on a molecular level that were previously not even formulated.

Many examples in this article also show that results depending completely on antibody specificity must be interpreted cautiously. First, antibodies, especially the monoclonal ones, often do not reveal the complete distribution of a molecule. Heterogeneity generated by pre- or posttranslational modifications can result in different epitopes being exposed on very similar, or identical, molecules. Second, monoclonal antibodies can bind to epitopes on different and sometimes unrelated molecules (*88*). The epitopes shared between classes of intermediate filaments (*89*) and the epitopes shared by the muscarinic and α_1-adrenergic receptors (*61*) are examples that probably do have functional bases. In the latter instance, the epitope was recognized only because sensitive assays exist for each receptor, and it is quite possible that all other receptors that act in a similar manner may contain this site. Until specificity is completely determined, a formidable proposition with rare molecules, the significance of the binding pattern of these antibodies will be uncertain. Clearly, the interpretation of immunological data is greatly strengthened by using antibodies to different epitopes on the same protein, and this will be required for many neural antigens. Antibodies to synthetic peptides corresponding to different sequences in the same protein have been used convincingly for this purpose (*90*). It has also been possible to use an antigen defined by a monoclonal antibody as an immunogen and obtain additional antibodies with the same binding pattern (*91*). Finally, many molecules of importance, such as rare trophic factors or channels, exist at such low concentrations that they will almost certainly not be detected by current screening procedures.

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