Surface Antigens of Brain Synapses: Identification of Minor Proteins Using Polyclonal Antisera

ANDREW MATUS, MEELIAN NG, GUNDULA PEHLING, MARCEL ACKERMANN, and KATHY HAUSER

Friedrich Miescher-Institut, 4002 Basel, Switzerland; and *Pharma Research Division, Ciba-Geigy Ltd., Basel, Switzerland. Dr. Ng's present address is Department of Biochemistry, University Kebangsan Malaysia, Kuala Lumpur, Malaysia.

ABSTRACT Antigenic proteins of brain synaptic plasma membranes (SPM) and postsynaptic densities (PSD) were characterized using antisera raised against SPM. Immunostaining of brain sections showed that the antigens were restricted to synapses, and electron microscopy revealed staining at both presynaptic terminals and PSDs. In primary brain cell cultures the antisera were also neuron-specific but the antigens were distributed throughout the entire neuronal plasma membrane, suggesting that some restrictive influence present in whole tissue is absent when neurons are grown dispersed. The antigenic proteins with which these antisera react were identified using SDS gel immunoblots. SPM and PSD differed from one another in their characteristic antigenic proteins. Comparison with amido-black stained gel blots showed that in both cases most of these did not correspond to known abundant proteins of SPM or PSDs revealed by conventional biochemical techniques. None of the antigens revealed by the polyclonal antisera were detected by any of a large series of monoclonal antibodies against SPM.

The synaptic region of the neuronal surface is involved in various specialized functions ranging from synaptic transmission to the establishment of specific interneuronal connections during development. Little is known about the nature of the membrane-bound molecules involved in these events, and for this reason a number of recent studies have been designed to identify and characterize synaptic plasma membrane (SPM) proteins. Much of this interest has centered on the postsynaptic density (PSD), a disk-shaped proteinaceous structure attached to the cytoplasmic face of the postsynaptic membrane (11, 33). PSDs can be isolated as enriched subcellular fractions and have been shown to possess a characteristic set of proteins that are different to those of SPM. Among the more abundant PSD components are cytoskeletal proteins such as tubulin (17, 26, 36) and actin (3, 6, 17, 23), calmodulin (10, 20, 37), and a junction-specific 51,000 Mr protein which is the major component of forebrain PSDs (1, 3, 6, 17, 26, 27) but appears to be absent from PSDs of cerebellum (5, 8).

In addition to these major components, PSDs also contain functional molecules such as neurotransmitter receptor-like binding sites for y-aminobutyric acid (GABA) (28) and glutamate (9, G. E. Fagg and A. Matus, unpublished observations). Minor components such as these, which are present in PSD preparations at very low abundance, may be of considerable physiological importance, but some means of characterization other than conventional protein detection techniques are necessary to study them. We have approached this problem by raising antisera against SPM and then using them to identify the antigenic SPM and PSD proteins with which they react. The results confirm the existence of characteristic and different sets of minor, antigenic SPM and PSD proteins, most of which do not correspond to previously described SPM PSD components. They also appear to represent synaptic surface antigens that have not been detected in previous studies using monoclonal antibodies.

MATERIALS AND METHODS

Subcellular Fractionation and Preparations of Antisera: SPMs were prepared from either rat cerebral cortex or cerebellum by the method of Jones and Matus (15). Rabbits were inoculated subcutaneously at
approximately monthly intervals with 1 mg of SPM protein suspended in 1 ml of 0.9% saline emulsified with an equal volume of Freund's complete adjuvant. This was continued until antisera were obtained that gave strong immunofluorescent staining on brain cryostat sections at 1:50 dilution. By solid phase immunoperoxidase assay against SPM all three antisera gave positive responses at dilutions of 1:5,000.

For the comparison of plasma membrane antigens from different tissues, an osmotically lysed P2 membrane fraction (12) was used. PSD-enriched fractions were prepared as previously described, and SDS gel electrophoresis and immunoblotting were performed as before (27). The apparent molecular weights of amido black and immunoperoxidase-stained polypeptides were calculated by reference to previously determined values for some of the same polypeptides (27). Gel blots, stained for protein with amido black or with antisera, were scanned by reflectance densitometry and peak areas measured by a Spectra-Physics integrator (Spectra-Physics, Inc., San Jose, CA).

Cell Cultures: Primary cell cultures were prepared from forebrains of newborn rats and grown at 5 x 10^6 cells/ml in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. For immunocytochemistry they were fixed with ethanol at -20°C for 15 min, treated with acetone at the same temperature for 5 min, and then air dried. Identical results were obtained when cultures were instead fixed with buffered 4% formaldehyde for 30 min.

Immunocytochemistry: Fixed cell cultures were incubated with phosphate-buffered saline (PBS = 50 mM phosphate buffer, pH 7.4, 150 mM NaCl) for 15 min. The cultures were stained for 30 min with antiserum diluted 1:100 with PBS, washed three times for 5 min with PBS, and then incubated with rhodamine-labeled goat anti-rabbit IgG (Nordic, Biogenzla Lemania, Lausanne). After washing with PBS they were mounted in glycerol/PBS (9:1). Immunoperoxidase staining of Vibratome sections from aldehyde-perfused rat brain was performed as previously described (27).

RESULTS

Immunocytological Evidence of Antisera Specificity

Three antisera were examined in this study. One was raised against SPM from rat cerebral cortex (anti-CTX) while the other two were against rat cerebellar SPM (anti-CBL1 and anti-CBL2). Each antiserum showed a high degree of selective reactivity toward brain synaptic membranes as judged by a variety of criteria. All three reacted strongly with tissue sections of brain (Fig. 1) and stained an array of punctate sites that in size and distribution resemble the pattern of synaptic staining seen with classical silver “bouton” stains (compare Fig. 1, a and c with 1, b and d). A similar pattern of staining with antisera against SPM seen in previous studies has also been attributed to synaptic staining (21, 25, 34).

In the cerebellum, small synapses throughout the molecular layer were stained whereas the other neuronal features, cell bodies, dendrites, and axons were unstained (Fig. 1). Glial cell bodies and their processes, such as the distinctive radial

![Figure 1](https://example.com/figure1.png) Comparison of the staining of brain sections with anti-SPM (anti-CTX, a and c) and with Bielschowsky silver stain for synaptic boutons (b and d). a and b show cerebellar Purkinje cells (1-3) with the overlying molecular layer to the left. c and d show the cerebellar granular layer (sg, synaptic glomeruli; gc, granule cell bodies). (a and b) x 500; (c and d) x 1,000.
Bergmann glial fibers in the molecular layer and astroglial endfeet on blood vessels, were also unstained by all three antisera. In the granule cell layer there was a strong punctate anti-SPM staining of the synaptic glomeruli (Fig. 1c) whose distribution corresponds to that of the silver bouton staining (Fig. 1d). As in the molecular layer, there was no staining of nonsynaptic features.

**Immunocytochemical Staining of Brain Cell Cultures**

When used as immunohistochemical stains of primary cell cultures from rat cerebral cortex, all three antisera reacted selectively with nerve cells and their processes without staining surrounding non-neuronal cells (Fig. 3, a and d). Most of the non-neuronal cells in these cultures were glial cells as judged by their staining with antisera against glial fibrillary acidic protein (GFAP) (Fig. 3e). In cultures stained with anti-GFAP, unstained neurons could be seen lying over the fluorescent filaments in the glial cells (Fig. 3f). Thus rabbit antibodies against synaptic membranes stain neurons, although in the same cultures an antibody against glial filaments leaves neurons unstained. This serves as further evidence that the anti-SPM sera are directed selectively against neuronal antigens.

The pattern of anti-SPM staining of cultured neurons was different in processes and cell bodies. Only the surfaces of neuronal processes were stained (Fig. 3c). One possible explanation for this could be that rabbit IgG cannot penetrate into the interior of the processes. However, in other experiments we have found that rabbit antisera against a variety of other proteins, including tubulin, microtubule-associated proteins, and neurofilaments, can stain these molecules within neuronal processes of cells in cultures from the same series as those used in this study (A. Matus and D. Beuret, unpublished observations). Thus the anti-SPM sera appear to be directed against neuronal components that are selectively associated with the surface membranes of neuronal processes. We also observed that at the neuronal cell body anti-SPM staining occurred throughout the cytoplasm but spared the nucleus (Fig. 3c). This indicates that the antibody can cross the plasma membrane of fixed neurons and also suggests that the antigenic proteins detected by our antibody are inserted into the plasma membrane close to the perikaryon.

**Ultrastructural Localization of Anti-SPM Staining**

To establish further the synaptic localization of anti-SPM staining, we processed antibody-stained brain sections for electron microscopy. Anti-CTX and anti-CBL2 gave staining patterns that were indistinguishable from one another (Fig. 4). In both cases the stain was concentrated in presynaptic terminals located throughout the neuropil but it was noticeable that unstained terminals occurred within the same field (Fig. 4a). Closer examination showed that within the presynaptic terminal the stain was present in the cytoplasm and on the cytoplasmic face of the presynaptic plasma membrane (Fig. 4b and c). Staining of the postsynaptic element with these two antisera was very much weaker than that found presynaptically. However, there was a faint yet reproducible staining of dendritic spines as judged against the absence of staining in neighboring glial cell processes (Fig. 4, b and c).

The other anti-SPM serum (anti-CBL1) gave a different staining pattern. Here the most strongly stained elements were the postsynaptic density and the surrounding cytoplasm, especially within the dendritic spine (Fig. 5). This antisera also gave faint but nevertheless distinct staining within dendrites. In addition the overall tissue background staining was also higher with anti-CBL1 suggesting that part of its activity may be directed against a generally distributed antigen, although this was not detectable in SDS gel immunoblot tests against membranes from various tissues or subcellular fractions.

**Identification of SPM and PSD Antigens**

The SPM and PSD proteins with which the anti-SPM sera react were identified using SDS gel blots. In this basic test each of the three antisera gave reproducible and characteristic results with several different SPM and PSD preparations. All of these data are summarized in Fig. 6, which shows the staining patterns obtained with each antisera on SPM and...
Figure 3  Primary cell cultures from rat cerebellum stained with anti-SPM sera by immunofluorescence. a, anti-CBL1; b, anti-CTX; c, anti-CTX (np, nerve cell processes). Arrowheads indicate neuronal cell bodies with stained cytoplasm but unstained nuclei. d, Anti-CBL2; e, anti-glial fibrillary acidic protein; f, anti-glial fibrillary acidic protein. The arrowhead indicates an unstained neuronal cell body. (a) × 125; (b and d) × 250; (c) × 2,500; (e) × 400; (f) × 1,500.
PSD gel blots and in Table I which shows the relative intensities of the stained bands.

All three antisera reacted with a large number of proteins in both the SPM and PSD fractions. Comparison of the antibody-stained bands with protein-stained gel blots showed that most of the prominent antigens do not correspond to abundant proteins. In PSD preparations there were 11 distinct high molecular weight antibody-reactive peptides (Table I) only one of which, with an $M_r$ of 280,000, appeared on gel blots stained for protein (Fig. 6, Am. bl.). In this high molecular weight range there was only one other abundant PSD protein with an $M_r$ of 180,000 (Fig. 6, Am. bl., lane 2; see also reference 27). It migrated close to an antigenic component of $M_r$ 176,000 but these two components reproducibly occupied different positions in adjacent stained strips from the same gel blot. Thus 10 of the main antigenic PSD proteins are minor components that are not detected by protein staining. These same components were absent from all other brain fractions tested by gel blotting (brain actomyosin, brain intermediate filaments, synaptic vesicles, and myelinated axons) except SPM, and in SPM they were markedly less concentrated than in PSD (Fig. 6). Conversely, the major PSD proteins of $M_r$'s 113,000, 50,000, and 46,000 were not strongly antigenic. Only the 70-kdalton-PSD peptide gave a distinct antigenic reaction with one of the antisera (Fig. 6, a-CTX, lane 2).

Comparison of the antibody- and protein-stained SPM gel blots is more difficult because of the complexity of the protein-stained pattern (Fig. 6, Am. bl., lane 7). However, a close comparison in individual cases suggests that the antigenic components being detected are not identical with protein-stained bands in the same region of the gel. The prominent anti-CTX-stained components with $M_r$'s of 49,000 and 40,000 are examples of antibody-stained bands whose size, shape, and position were distinctly different to the protein-stained bands in the same molecular weight range.
DISCUSSION

Many of the potentially most interesting molecules of the synaptic surface are either known to be present in low molecular abundance, such as neurotransmitter receptors, or may be expected to be present in low concentration, such as surface signal molecules for determining histotypic relationships between different cell types. In addition to their low absolute amounts, these same molecules co-exist with much more abundant proteins in an organelle structure that, in the case of the PSD, is insoluble under any conditions other than those that simultaneously denature the proteins as they are extracted.

Antibodies offer an alternative means of working with material so refractory to the straightforward biochemical approach of fractionation and enrichment of individual molecules of interest. One advantage to using antibodies is that some major components of the synaptic structure may be only weakly antigenic, as the major forebrain PSD protein, PSD-50, has proved to be (8, our unpublished observations), whereas other, quantitatively minor components, may be strongly antigenic. Thus immunizing an animal with whole membranes may yield a population of serum antibodies recognizing a quite different set of SPM or PSD proteins to those detected by conventional biochemical techniques. This expectation is borne out by the results of the present study that show that many of the SPM and PSD polypeptides recognized by our antisera are indeed minor components distinct from the more abundant proteins of these structures (Fig. 6, compare also references 1, 3, 6, 8, 26, 27, 30, 35).

Because our initial inoculum consisted of membrane fragments, the polyclonal antisera obtained may be expected to contain individual antibodies against molecules associated with many individual types of synapses. Having demonstrated as we have here, that such antisera do indeed contain antibodies against SPM and PSD, it should then be possible, using hybridoma technology, to isolate replicating lymphocyte clones producing unique antibodies reacting with specific proteins. In principle, this ability to "clone-out" individual antibodies starting with complex mixtures of antigens is the decisive advantage that the monoclonal antibody technique has to offer for studying synaptic structure (13, 14).

In practice, we now recognize several restrictions that limit our use of the monoclonal approach. In our original studies we inoculated mice with rat brain SPM and recovered several hundred monoclones secreting antibodies against neural antigens (13). Not one of these has proved to be directed against PSDs. Further experience has produced similar results; in successive fusions we repeatedly obtain hybridomas secreting antibodies against the same "dominant" antigens with further novel antigens emerging at a rate of only a few percent over several thousand hybridomas we have analyzed (B. Riederer and A. Matus, unpublished observations). One possible explanation for this may be related to the comparative number of spleen cells contributing serum antibodies in the animal and the number of Ig-secreting hybridomas formed from the

FIGURE 5 Electron micrograph of rat cerebral cortex stained with anti-CBL1. The terminals of two synapses (t) and their postsynaptic spines (sp) are identified. Arrowheads indicate PSDs (den, dendrite segment). Bar, 0.2 μm.

FIGURE 6 Nitrocellulose blots of SDS gels in which proteins of SPM (strips labeled 1) and PSD (strips labeled 2) had been separated. Pairs of blots are shown stained with antiserum against rat cerebral cortex SPM (a-CTX) or rat cerebellar SPM (a-CBL1, a-CBL2). For comparison, further blots from the same gels are shown stained for protein with amido black (Am. bl.). Calculated molecular weights X 10^-3 are indicated.
spleen of the same animal in a hybridoma fusion experiment. Of the 10^7 spleen cells put into a myeloma fusion only a small proportion will form Ig-secreting hybrids (38). In our experience several hundred primary secreting clones is the maximum that can be expected out of some 2,000 viable hybridoma colonies formed in even the best fusion. Considering that the antibody repertoire of an inbred mouse is in the range 1 to 5 x 10^12 with some 1 to 8 x 10^12 different antibodies being potentially available for a single antigenic determinant (18, 19), it is clear that very little of the immunological potential is tapped by making monoclonal antibodies.

This problem is particularly acute for complex organelles such as synaptic membranes where it is inconceivable that a range of monoclonal antibodies could be developed to even remotely challenge the richness of antibody specificities contained in a single easily-derived polyclonal antiserum. The essential point is that the two methods are suited for different, complimentary purposes; monoclonal antibodies being excellent specific reagents for single antigens whereas polyclonal antisera excel as reagents where antibody diversity is the prime requirement.

The value of polyclonal antisera is not restricted simply to detecting antigens. They are also valuable for studying the ways in which a family of related antigens behaves as a group. This has already been illustrated with respect to synaptic surface antigens in past studies which showed that antigenic synaptosomal and SPM antigens are concentrated at the synapse (21, 25, 34) suggesting that despite the demonstrable fluidity of the synaptic membranes, (2, 24) there are local influences that restrict the mobility of membrane-bound components at the synaptic surface (25, 34).

At least some of this restriction can be accounted for by antigens bound to the PSD, where surface-bound molecules are apparently immobilized (7, 29), and some of the synaptic antigens studied in the past have been localized by immunoelectron microscopy to the PSD (22, 32, 31). However, in this study we found that the most abundant of our synaptic antigens were associated with SPM rather than PSD, and immunoelectron microscopy showed them to be located pre-synaptically. This suggests further that some kind of restrictive influence operates on many SPM proteins to Marshall them at the synaptic area of the neuronal plasma membrane.

A very different situation was found in brain cell cultures. Instead of being limited to synapses, the antigens in cultured neurons were spread evenly over the entire cell surface. Thus the restrictive influence that maintains these antigenic proteins at synaptic areas of neurons in whole brain appears to be absent in dispersed cell cultures. This suggests that some aspect of histotypic organization is responsible for the restrictive localization of synaptic proteins in whole brain.

The antiserum against glial fibrillary acidic protein used in this study was generously donated by Dr. Doris Dahl.

Received for publication 15 December 1982, and in revised form 17 August 1983.

REFERENCES

1. Banker, G. L., Churchill, and C. W. Cotman. 1974. Proteins of the postsynaptic density. J. Cell Biol. 63:462-465.
2. Bettiger, H., and H.-P. Schnell. 1974. Binding of concanavalin A and ricin to synaptic junctions of the rat brain. Nature (Lond.). 249:270-271.
3. Blomberg, F., R. S. Cohen, and P. Siekervitz. 1977. The structure of postsynaptic densities isolated from dog cerebral cortex. II. Characterization and arrangement of some of the major proteins within the structure. J. Cell Biol. 74:200-225.
4. Carlin, R. K., D. C. Bartlet, and P. Siekervitz. 1983. Identification of fodrin as a major calmodulin-binding protein in postsynaptic density preparations. J. Cell Biol. 96:443-448.
5. Carlin, R. K., D. J. Grab, R. S. Cohen, and P. Siekervitz. 1980. Inclusion and characterization of postsynaptic densities from various brain regions enrichment of different types of postsynaptic densities. J. Cell Biol. 86:831-843.
6. Cohen, R. S., F. Blomberg, K. Beirne, and P. Siekervitz. 1977. The structure of postsynaptic densities isolated from dog cerebral cortex. I. Overall morphology and protein composition. J. Cell Biol. 74:181-203.
7. Cotman, C. W., and D. Taylor. 1974. Localization and characterization of concanavalin A receptors in the synaptic cleft. J. Cell Biol. 62:236-242.
8. Flanagan, S., B. Yost, and H. F. W. R. 1982. Putative 51,000-Mr. protein marker for postsynaptic densities is virtually absent in cerebellum. J. Cell Biol. 94:743-748.
9. Foster, A. N. C., E. E. Mena, G. E. Fagg, and C. W. Cotman. 1981. Glutamate and aspartate binding sites are enriched in synaptic junctions isolated from rat brain. J. Neuroscience. 7:623-625.
10. Grab, D. J., R. K. Carlin, and P. Siekervitz. 1980. The presence and possible function of calmodulin in isolated cerebral cortex post-synaptic densities. Ann. N.Y. Acad. Sci. 356:21-72.
11. Gray, E. C. 1959. Axo-somatic and axo-dendritic synapses of cerebral cortex. An electron microscopic study. J. Anat. 93:420-423.
12. Gray, E. G., and V. P. Whitaker. 1962. The isolation of nerve endings from brain: an electron microscopic study of cell fragments derived by homogenization and centrifugation. J. Anat. 96:79-88.
13. Hawkes, R. E., and A. Matus. 1982. Monoclonal antibodies identify novel neural antigens. Proc. Natl. Acad. Sci. USA. 79:2410-2414.
14. Hawkes, R. E., and N. A. Matus. 1982. Using antibodies to probe brain development. In Neurotransmitter Interaction and Categorization. H. F. Bradford, editor. Plenum Press, Inc., New York. 39-51.
15. Jones, D. H., and A. M. C. 1981. Isolation of synaptic plasma membrane from brain by combined flotation-sedimentation density gradient centrifugation. Biochem. Biophys. Acta. 65:276-287.
16. Jorgensen, O. S., and E. Bock. 1974. Brain-specific synaptosomal membrane proteins demonstrated by crossed immunoelectrophoresis. J. Neurochem. 23:879-880.
17. Kelly, P. T., and C. W. Cotman. 1978. Synaptic proteins: characterization of tubulin and actin and identification of a distinct postsynaptic density protein. J. Cell Biol. 79:173-183.
18. Kreth, H. W., and A. R. Williamson. 1973. The extent of diversity of anti-hapten antibodies in inbred mice: anti-NIP (4-hydroxy-4-imidazolyl)-3-nitrophenacyl) antibodies in CBA/H mice. Eur. J. Immunol. 3:141-147.
19. Köhler, G. 1976. Frequency of precursors cells against the enzyme β-galactosidase. An estimate of the BALB/c strain antibody repertoire. Eur. J. Immunol. 6:340-347.
20. Lin, C. T. J. R. Bedman, B. R. Brinkley, and A. M. W. 1980. Localization of calmodulin in rat cerbellum by immunoelectron microscopy. J. Cell Biol. 85:473-480.
21. Livoti, B. G., J. A. P. Rostas, P. L. Jeffrey, and L. Austin. Antigenity of isolated synaptosomal membranes. Exp. Neurol. 43:330-338.
22. Matus, A. 1975. Immunohistochemical demonstration of antigen associated with the postsynaptic lattice. J. Neurocytol. 4:47-53.
23. Matus, A., M. Ackermann, G. Pehling, H. R. Byers, and K. Fujisawa. 1982. High actin concentrations in brain dendritic spines and postsynaptic densities. Proc. Natl. Acad. Sci. USA. 79:7590-7594.
24. Matus, A., S. de Petris, and M. C. Raff. 1973. Mobility of concanavalin A receptors in myelin and synaptic membranes. Nat. New Biol. 244:278-280.
25. Matus, A., D. H. Jones, and S. Mughal. 1976. Restricted distribution of synaptic antigens in the neuronal membrane. Brain Res. 103:171-175.
26. Matus, A., and D. H. Taff-Jones. 1978. Morphological and molecular composition of isolated post-synaptic junctional structures. Proc. R. Soc. Lond. B Biol. 203:135-151.
27. Matus, A., G. Pehling, M. Ackermann, and J. Maeder. 1980. Brain postsynaptic densities: their relationship to glial and neuronal filaments. J. Cell Biol. 87:346-359.
28. Matus, A., G. Pehling, and D. Wilkinson. 1981. γ-Aminobutyric acid receptors in brain postsynaptic densities. J. Neurobiol. 12:67-73.
29. Matus, A., and B. B. Walters. 1976. Type I and 2 synaptic junctions: differences in distribution of concanavalin A binding sites and stability of the junctional adhesion. Brain Res. 108:249-256.
30. Morgan, J. G., L. S. Wolfe, P. Mandel, and G. Gombrs. 1971. Isolation of plasma membranes from rat brain. Biochim. Biophys. Acta. 241:737-751.
31. Nieto-Sampedro, M. N., C. M. Bussineau, and C. W. Cotman. 1981. Postsynaptic density antigens. Preparation and characterization of an antiserum against postsynaptic densities. J. Cell Biol. 90:675-686.
32. Orosz, A., J. Hamori, A. Falus, E. Madarasz, I. Lakos, and G. Adam. 1973. Specific antibody fragments against the postsynaptic web. Nat. New Biol. 245:16-19.
33. Palay, S. L. 1958. The morphology of synapses in the central nervous system. Exp. Cell Res. 1:275-293. (Suppl.)
34. Rosas, J. A. P., and P. L. Jeffrey. 1975. Restricted mobility of neuronal membrane antigens. Neurosci. Lett. 1:47-53.
35. Wang, Y.-J., and H. R. Mahler. 1976. Topography of the synaptosomal membrane. J. Cell Biol. 71:639-658.
36. Walters, B. B., and A. 1. Matus. 1975. Tubulin in post-synaptic junctional lattice. Nature (Lond.) 257:496-498.
37. Wood, J. G., R. Wallace, J. Whitaker, and W. Y. Cheung. 1980. Immunocytochemical localization of calmodulin and a heat-labile calmodulin-binding protein (C-M-BPs) in basal ganglia from mouse brain. J. Cell Biol. 84:66-76.
38. Yeltos, D. E., D. H. Margules, B. Diamond, and M. D. Scharff. 1980. Plasmacytomas and hybridomas. Development and applications. In Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analysis. R. H. Kennett, H. Kennet, T. J. McKearn, and K. B. Bechtol, editors. Plenum Press, New York. pp. 3-17.