Neuroimmunology

Edited by
JEREMY BROCKES

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JEREMY BROCKES
California Institute of Technology
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Contributors

M. JOHN ANDERSON
Department of Embryology
Carnegie Institution of Washington
Baltimore, Maryland

COLIN J. BARNSTABLE
Department of Neurobiology
Harvard Medical School
Boston, Massachusetts

ELLEN K. BAYNE
Department of Embryology
Carnegie Institution of Washington
Baltimore, Maryland

HARVEY CANTOR
Department of Pathology
Harvard Medical School and Sidney Farber Cancer Institute
Boston, Massachusetts

LINDA L. Y. CHUN
Department of Pathology
Harvard Medical School and Sidney Farber Cancer Institute
Boston, Massachusetts

DOUGLAS M. FAMBROUGH
Department of Embryology
Carnegie Institution of Washington
Baltimore, Maryland

JOHN M. GARDNER
Department of Embryology
Carnegie Institution of Washington
Baltimore, Maryland

ZACH W. HALL
Division of Neurobiology
Department of Physiology
University of California
San Francisco, California

REGIS B. KELLY
Department of Biochemistry and Biophysics
University of California
San Francisco, California

WILLIAM D. MATTHEW
Department of Physiology
University of California
San Francisco, California

RHONA MIRSKY
MRC Neuroimmunology Group
Department of Zoology
University College London
London, England

MICHAEL B. A. OLDSTONE
Department of Immunopathology
Scripps Clinic and Research Foundation
La Jolla, California

ERIC OUTWATER
Department of Physiology
University of California
San Francisco, California

LOUIS F. REICHARDT
Department of Physiology
University of California
San Francisco, California

RICHARD L. ROTUNDO — Department of Embryology
Carnegie Institution of Washington
Baltimore, Maryland

MELITTA SCHACHNER — Department of Neurobiology
University of Heidelberg
Heidelberg, Federal Republic of Germany

LARISA TSAVALER — Department of Physiology
University of California
San Francisco, California

ERIC WAKSHULL — Department of Embryology
Carnegie Institution of Washington
Baltimore, Maryland

Preface

Neuroimmunology could be defined as the application of immunological methods to problems in neurobiology but such a definition is so all-encompassing as to be unhelpful. It is not a precisely circumscribed discipline but it seems worthwhile at the outset to point to three of the major areas of activity.

One rather early use of the term was in connection with studies on the immune response to antigens in the nervous system. This includes topics such as autoimmunity in the central and peripheral nervous systems, the response to neural tumors or viral infections, and the immunopathology of such processes. Although not at the forefront of the currently fashionable preoccupation with neuroimmunology, this area continues to be a vital and interesting one from both clinical and basic perspectives.

A second very active area is the exploitation of antibodies to identified components of neural cells, and in particular to those molecules involved as neurotransmitters, in transmitter synthesis and breakdown, and as synaptic receptors. The immunohistochemical detection of these antigens has led to new insights into the functional organization of the nervous system, and reference to such studies is almost a *sine qua non* for discussions of most central and peripheral synapses.

The third area has its intellectual and practical antecedents in the discipline called cellular immunology—the study of different classes of lymphocyte and their functional interactions. As discussed in this volume by Chun and Cantor, the considerable advances in cellular immunology in the last fifteen years have in large part depended on the use of antisera to marker antigens, generally on the cell surface, which are confined to particular subpopulations of lymphocytes. A distinctive methodology has developed around the derivation and assay of such antisera, and their exploitation for identifying and purifying the different

classes of T and B cells. The derivation of these reagents after immunizing with complex mixtures of antigens has been revolutionized by hybridoma technology. It is important to note that many of the marker antigens which have been so valuable in cellular immunology are molecules whose function is unknown, at least initially. The study of cellular heterogeneity among neurons and glia is, at one level or another, a basic preoccupation of neurobiologists. It is therefore a natural development to use the methodology of cellular immunology firstly to reinforce or refine traditional classifications based largely on anatomy or transmitter type, and secondly to seek new identities and differences between cells as an expression of fundamental aspects of development and organization.

This collection of essays reflects all of these interests. The authors' viewpoints are different, varying from studies on components of a particular synapse or cell surface, to an analysis of cell markers in the nervous system or particular areas of the brain, and to considerations of autoimmunity and the immune response that were referred to above. Together they provide a strong case for why neuroimmunology is currently so interesting and exciting, and also why it seems to be so promising for tackling some of the traditionally intractable problems in neuroscience.

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Jeremy P. Brockes
Pasadena, California

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1

Immunology of the Neuromuscular Junction

REGIS B. KELLY and ZACH W. HALL

1. INTRODUCTION

Although the vertebrate neuromuscular junction has surely been the most intensively studied synapse in the nervous system, we know remarkably little about its structure and function at a molecular level. One simple reason for this is that the junction forms only a miniscule part of the total muscle tissue, and methods have not been devised for its purification as an intact structure. Isolation of any molecular component of the synapse is thus a formidable task. The only protein that has been extensively purified from the neuromuscular junction is the acetylcholine receptor (AChR); even in this case, the small amount of protein that can be obtained limits severely the kinds of experiments that can be done.

Because the neuromuscular junction is inaccessible to biochemical analysis, antibodies have become, and will continue to be, unusually important tools in its investigation. Components of the junction that cannot be detected or localized by direct assay can be identified and assayed by immunohistochemical methods. Two factors increase the power of this approach. The first one is the homology between the

REGIS B. KELLY · Department of Biochemistry and Biophysics, University of California, San Francisco, Calif. 94143. ZACH W. HALL · Division of Neurobiology, Department of Physiology, University of California, San Francisco, Calif. 94143.

vertebrate neuromuscular junction and the synapses in the electric organs of various fish. Because the electric organs receive dense innervation and are abundantly available, antibodies can be made to macromolecules purified from these tissues and then used to study the neuromuscular junction. This approach has been most thoroughly applied with respect to the AChR. The ready availability of purified receptor from *Torpedo* has made the production of antibodies to it a routine matter. These sera cross-react with the mammalian receptor; their use has been of enormous importance in advancing our understanding of the pathophysiology of the neuromuscular junction.

The second important factor is the recent development of the monoclonal technique. Antibodies can now be made to unidentified or partially purified components of the junction. Beyond simply identifying the macromolecules that are present, monoclonal antibodies (mAbs) provide extremely precise and well-defined reagents that can be used to intervene in functional or developmental processes or to define the role of particular parts of macromolecules. The potentiality of this approach is only beginning to be realized.

In this article, we describe the elements of the neuromuscular junction to which antibodies are currently available. We also emphasize the various uses to which these antibodies can be put. In some cases, they have been used to identify molecular components of the synapse for which no other assay exists, or to show that molecules found elsewhere in the body are also present at the synapse. They have been useful in structural studies of synaptic molecules, even sometimes as inhibitors of function. They have been used as markers of synaptic function and to purify synaptic components. They can be used as developmental markers. The conclusion we wish to draw is that many molecular aspects of the neuromuscular junction which were once considered unapproachable are now accessible by immunological methodology.

2. STRUCTURE OF THE NEUROMUSCULAR JUNCTION

The three parts of the neuromuscular junction (Fig. 1) to which we will pay attention are the nerve terminal, the synaptic cleft, and the postsynaptic membrane with its underlying structures. The presynaptic terminal is specialized for the synthesis, storage, and release of the transmitter ACh, and for the uptake of its precursor, choline. Morphologic specializations include synaptic vesicles and specialized sites around which vesicles are clustered on the membrane facing the muscle surface. These regions, called active zones (Couteaux and Pecot-De-

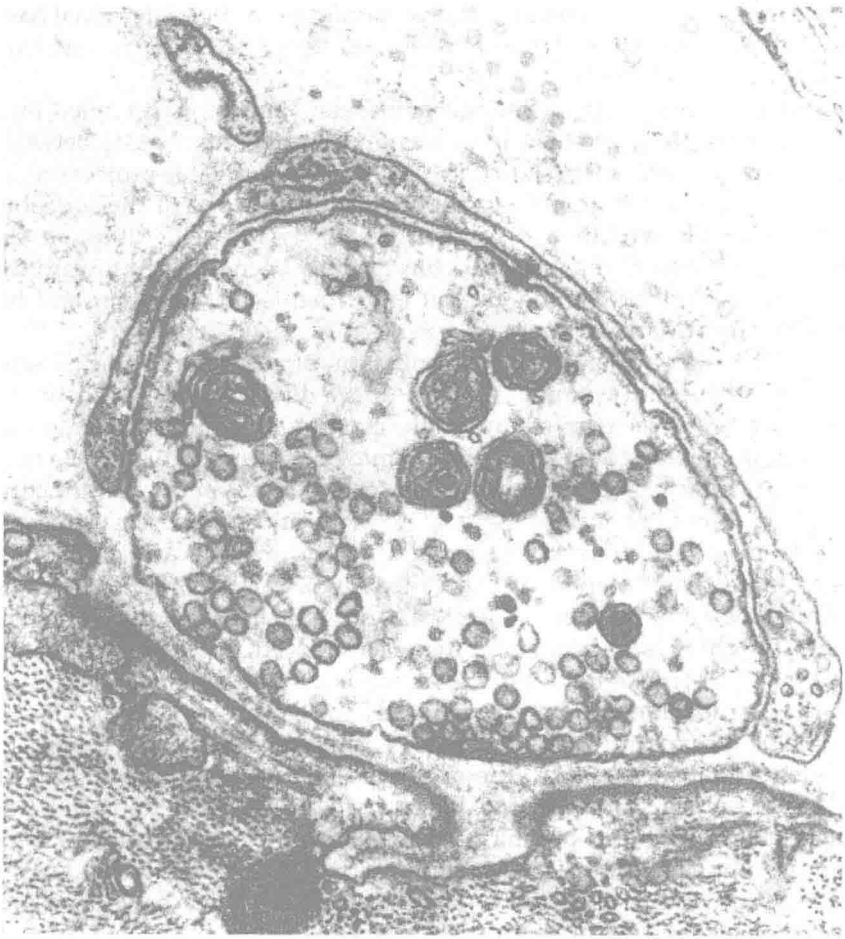

Figure 1. Electron micrograph of cross-sectional frog neuromuscular junction. (Reprinted from Sanes *et al.*, 1980.)

chavissine, 1970), are thought to be the sites of transmitter release (Heuser *et al.*, 1979) and are positioned exactly opposite the folds in the postsynaptic membrane. In thin section, they are characterized by a small amount of dense material on the inside of the membrane. Freeze-fracture experiments demonstrate the active zones to contain a double row of particles that extend across the terminal. The motor nerve terminal is known to have a high density of voltage-sensitive calcium channels, and it has been suggested that the particles may correspond to

these molecules. The structure and properties of the nerve terminal have recently been reviewed (Heuser and Reese, 1979; Ceccarelli and Hurlbut, 1980; Kelly *et al.*, 1979).

The synaptic cleft of the neuromuscular junction is occupied by a basal lamina (BL) that ensheathes each muscle fiber and passes between nerve and muscle at the junction. Within the endplate, projections of the BL extend into the postsynaptic folds; at the edge of the junction, the muscle BL joins that of the overlying Schwann cell. Although virtually ignored for years, attention has recently been directed toward the BL of muscle fibers by the elegant experiments of McMahan and his collaborators (Marshall *et al.*, 1977; Sanes *et al.*, 1978; Burden *et al.*, 1979). They have shown that during regeneration of adult nerve and muscle, the BL at the old synaptic site can direct differentiation both of the presynaptic nerve terminal and of the postsynaptic muscle membrane.

Very little is known about the chemical structure of the muscle fiber BL, particularly at the synapse which represents such a small fraction of the muscle fiber surface. By analogy with other BLs it is assumed to consist of collagen, glycoproteins, and glycosaminoglycans. In contrast to pre- and postsynaptic structures from which components can often be identified and purified on the basis of enzymatic or binding activities, almost nothing is known about the macromolecular components of the synaptic BL. Thus immunological methods assume unusual importance in the identification and study of these molecules.

One component of the synaptic BL has been identified: AChE. The evidence that the AChE in the synaptic cleft is associated with the BL is based on the studies of McMahan *et al.* (1978) using damaged frog muscle in which the plasma membrane of the muscle cell has been removed by phagocytosis. After damage only the BL remains, yet AChE activity can still be detected at old endplates. Antisera have been prepared to purified AChE from several sources.

The only component of the postsynaptic membrane to be so far identified is the AChR which binds the ACh released from presynaptic terminals. This binding reaction causes the transient opening (ca. 1 ms) of a transmembrane channel that is selectively permeable to the cations, sodium, potassium, and calcium. In muscle, where the postsynaptic membrane is thrown into elaborate folds, the AChR occurs on the crests of the folds, at a density of about 2×10^4 toxin-binding sites per square micrometer (Fertuck and Salpeter, 1974). In the electric organ of *Torpedo* the receptors are packed at a similarly high density: the entire surface of the electroplax is continuously innervated and is covered with AChRs. These have been visualized as particles of about 8 nm diameter by negative staining, by freeze fracture, and by freeze fracture followed by deep etching (Heuser and Salpeter, 1979). After negative staining, the