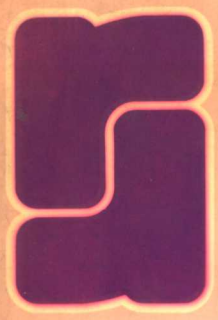

Receptors and Recognition

Series B Volume 17

Monoclonal Antibodies to Receptors

Probes for Receptor Structure and Function

Edited by
M. F. Greaves

Receptors and
Recognition

Series B Volume 17

Monoclonal Antibodies to Receptors

Probes for Receptor Structure and Function

Edited by
M. F. Greaves

Leukaemia Research Fund Centre
Institute for Cancer Research,
London, U.K.

LONDON NEW YORK
CHAPMAN AND HALL

First published 1984 by
Chapman and Hall Ltd
11 New Fetter Lane, London EC4P 4EE

Published in the USA by
Chapman and Hall
733 Third Avenue, New York NY 10017

Printed in Great Britain at the
University Press, Cambridge

ISBN 0 412 25330 5

British Library Cataloguing in Publication Data

Monoclonal antibodies to receptors.——(Receptors and recognition. Series B; V.17)
1. Antibodies, Monoclonal 2. Cell receptors
I. Greaves, M.F. II. Series
596'.0293 QR186.85

ISBN 0-412-25330-5

Library of Congress Cataloging in Publication Data

Main entry under title:

Monoclonal antibodies to receptors.

(Receptors and recognition. Series B; v. 17)
Bibliography: p.
Includes index.
1. Antibodies, Monoclonal. 2. Cell receptors.
3. Hormone receptors. 4. Neurotransmitter receptors.
I. Greaves, M. F. (Melvyn F.), 1941– . II. Series.
[DNLM: 1. Antigen-antibody reactions. 2. Antibodies, Monoclonal. 3. Receptors, Immunologic.
W1 RE107MA v. 17/QW 570 A6246]
QR186.85.A58 1984 596'.087 84-4976
ISBN 0-412-25330-5

Receptors and Recognition

General Editors: P. Cuatrecasas and M.F. Greaves

About the series

Cellular Recognition – the process by which cells interact with, and respond to, molecular signals in their environment – plays a crucial role in virtually all important biological functions. These encompass fertilization, infectious interactions, embryonic development, the activity of the nervous system, the regulation of growth and metabolism by hormones and the immune response to foreign antigens. Although our knowledge of these systems has grown rapidly in recent years, it is clear that a full understanding of cellular recognition phenomena will require an integrated and multidisciplinary approach.

This series aims to expedite such an understanding by bringing together accounts by leading researchers of all biochemical, cellular and evolutionary aspects of recognition systems. This series will contain volumes of two types. First, there will be volumes containing about five reviews from different areas of the general subject written at a level suitable for all biologically oriented scientists (Receptors and Recognition, series A). Secondly, there will be more specialized volumes (Receptors and Recognition, series B), each of which will be devoted to just one particularly important area.

Receptors and Recognition

Series A

Published

Volume 1 (1976)

M.F. Greaves (London), Cell Surface Receptors: A Biological Perspective

F. Macfarlane Burnet (Melbourne), The Evolution of Receptors and Recognition in the Immune System

K. Resch (Heidelberg), Membrane Associated Events in Lymphocyte Activation

K.N. Brown (London), Specificity in Host-Parasite Interaction

Volume 2 (1976)

D. Givol (Jerusalem), A Structural Basis for Molecular Recognition: The Antibody Case

B.D. Gomperts (London), Calcium and Cell Activation

M.A.B. de Sousa (New York), Cell Traffic

D. Lewis (London), Incompatibility in Flowering Plants

A. Levitski (Jerusalem), Catecholamine Receptors

Volume 3 (1977)

J. Lindstrom (Salk, California), Antibodies to Receptors for Acetylcholine and other Hormones

M. Crandall (Kentucky), Mating-Type Interaction in Micro-organisms

H. Furthmayr (New Haven), Erythrocyte Membrane Proteins

M. Silverman (Toronto), Specificity of Membrane Transport

Volume 4 (1977)

M. Sonenberg and A.S. Schneider (New York), Hormone Action at the Plasma Membrane: Biophysical Approaches

H. Metzger (NIH, Bethesda), The Cellular Receptor for IgE

T.P. Stossel (Boston), Endocytosis

A. Meager (Warwick) and R.C. Hughes (London), Virus Receptors

M.E. Eldefrawi and A.T. Eldefrawi (Baltimore), Acetylcholine Receptors

Volume 5 (1978)

P.A. Lehmann (Mexico), Stereoselective Molecular Recognition in Biology

A.G. Lee (Southampton, U.K.), Fluorescence and NMR Studies of Membranes

L.D. Kohn (NIH, Bethesda), Relationships in the Structure and Function of Receptors for Glycoprotein Hormones, Bacterial Toxins and Interferon

Volume 6 (1978)

J.N. Fain (Providence, Rhode Island), Cyclic Nucleotides

G.D. Eytan (Haifa) and B.J. Kanner (Jerusalem), Reconstitution of Biological Membranes

P.J. O'Brien (NIH, Bethesda), Rhodopsin: A Light-sensitive Membrane Glycoprotein

Index to Series A, Volumes 1–6

Series B

Published

The Specificity and Action of Animal Bacterial and Plant Toxins (B1)
edited by P. Cuatrecasas (Burroughs Wellcome, North Carolina)
Intercellular Junctions and Synapses (B2)
edited by J. Feldman (London), N.B. Gilula (Rockefeller University, New York) and J.D. Pitts (University of Glasgow)
Microbial Interactions (B3)
edited by J.L. Reissig (Long Island University, New York)
Specificity of Embryological Interactions (B4)
edited by D.R. Garrod (University of Southampton)
Taxis and Behavior (B5)
edited by G.L. Hazelbauer (University of Uppsala)
Bacterial Adherence (B6)
edited by E.H. Beachey (Veteran's Administration Hospital and University of Tennessee, Memphis, Tennessee)
Virus Receptors Part 1 Bacterial Viruses (B7)
edited by L.L. Randall and L. Philipson (University of Uppsala)
Virus Receptors Part 2 Animal Viruses (B8)
edited by K. Lonberg-Holm (Du Pont, Delaware) and L. Philipson (University of Uppsala)
Neurotransmitter Receptors Part 1 Amino Acids, Peptides and Benzodiazepines (B9)
edited by S.J. Enna (University of Texas at Houston) and H.I. Yamamura (University of Arizona)
Neurotransmitter Receptors Part 2 Biogenic Amines (B10)
edited by H.I. Yamamura (University of Arizona) and S.J. Enna (University of Texas at Houston)
Membrane Receptors: Methods for Purification and Characterization (B11)
edited by S. Jacobs and P. Cuatrecasas (Burroughs Wellcome, North Carolina)
Purinergic Receptors (B12)
edited by G. Burnstock (University College, London)
Receptor Regulation (B13)
edited by R.J. Lefkowitz (Duke University Medical Center, North Carolina)
Histocompatibility Antigens: Structure and Function (B14)
edited by P. Parham (Stanford University School of Medicine, California) and J. Strominger (Harvard University, Massachussetts)
Receptor-Mediated Endocytosis (B15)
edited by P. Cuatrecasas (Burroughs Wellcome, North Carolina) and T. Roth (University of Maryland, Baltimore County)
Genetic Analysis of the Cell Surface (B16)
edited by P. Goodfellow (Imperial Cancer Research Fund, London)

Contributors

Maurizio Bifulco, Laboratory of Biochemical Pharmacology, National Institutes of Health, Bethesda, Maryland, U.S.A.

Pedro Cuatrecasas, Wellcome Research Laboratories, Research Triangle Park, North Carolina, U.S.A.

Michele L. De Luca, Laboratory of Biochemical Pharmacology, National Institutes of Health, Bethesda, Maryland, U.S.A.

Joel M. Depper, Metabolism Branch, National Cancer Institute, Bethesda, Maryland, U.S.A.

Claire M. Fraser, Department of Molecular Immunology, Roswell Park Memorial Institute, Buffalo, New York, U.S.A.

Sara Fuchs, Department of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel.

Melvyn F. Greaves, Membrane Immunology Laboratory Leukaemia Research Fund Centre, Institute for Cancer Research, London, U.K.

Warner C. Greene, Metabolism Branch, National Cancer Institute, Bethesda, Maryland, U.S.A.

Evelyn F. Grollman, Laboratory of Biochemical Pharmacology, National Institutes of Health, Bethesda, Maryland, U.S.A.

Steven Jacobs, Wellcome Research Laboratories, Research Triangle Park, North Carolina, U.S.A.

Hana Kanety, Department of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel.

Leonard D. Kohn, Laboratory of Biochemical Pharmacology, National Institutes of Health, Bethesda, Maryland, U.S.A.

Frederick C. Kull Jr, Wellcome Research Laboratories, Research Triangle Park, North Carolina, U.S.A.

Irit Lax, Department of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel.

Warren J. Leonard, Metabolism Branch, National Cancer Institute, Bethesda, Maryland, U.S.A.

Tovia Libermann, Department of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel.

Daria Mochly-Rosen, Department of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel.

Bruno Moncharmont, Wellcome Research Laboratories, Research Triangle Park, North Carolina, U.S.A.

Roland A. Newman, Department of Cancer Biology, Salk Institute for Biological Studies, San Diego, California, U.S.A.

Indu Parikh, Wellcome Research Laboratories, Research Triangle Park, North Carolina, U.S.A.

Gordon D. Ross, Department of Medicine and Department of Microbiology – Immunology, University of North Carolina, Chapel Hill, North Carolina, U.S.A.

Joseph Schlessinger, Department of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel.

Claudio Schneider, Membrane Immunology Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, U.K.

Alain B. Schreiber, Institute of Biological Sciences, Syntex Research, Palo Alto, California, U.S.A.

Miry C. Souroujon, Department of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel.

A. Donny Strosberg, Molecular Immunology, Institut Jacques Monod, C.N.R.S. and University of Paris VII, Paris, France.

Cox Terhorst, Laboratory of Molecular Immunology, Dana Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts, U.S.A.

Donatella Tombaccini, Laboratory of Biochemical Pharmacology, National Institutes of Health, Bethesda, Maryland, U.S.A.

Ian S. Trowbridge, Department of Cancer Biology, Salk Institute for Biological Studies, San Diego, California, U.S.A.

William A. Valente, Laboratory of Biochemical Pharmacology, National Institutes of Health, Bethesda, Maryland, U.S.A.

Thomas A. Waldmann, Metabolism Branch, National Cancer Institute, Bethesda, Maryland, U.S.A.

Yosef Yarden, Department of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel.

Preface

Receptor specific antibodies are excellent probes for a wide range of biological investigations on receptor structure and function. The hybridoma technology (Kohler and Milstein, 1975) has inevitably had a major impact on this field with most of the better known receptors now identified with monoclonal antibodies. This volume of the *Receptors and Recognition* series provides reviews of recent developments in this field and emphasizes in particular the new opportunities afforded by the judicious application of monoclonal reagents. It is assumed that most readers will be familiar with the now fairly routine methods of cell fusion, hybridoma cloning and selection for producing monoclonal antibodies and so few details of the basic technical procedures are described. Several good reviews on this topic are however available (see Galfre and Milstein, 1981; Goding, 1980; Yelton and Scharf, 1981; McMichael and Fabre, 1982).

By no means all vertebrate receptor species are discussed here; omissions include antibodies to low density lipoprotein receptors (Beisiegel *et al.*, 1981; Kita *et al.*, 1981), prolactin and growth hormone receptors (Friesen *et al.*, 1982; Simpson *et al.*, 1983) and the hepatocyte asialoglycoprotein receptor (Schwartz *et al.*, 1981; Harford *et al.*, 1982). Nevertheless the coverage is comprehensive and critical and the individual chapters provided illustrate vividly the rapid progress being made. In addition to monoclonal antibodies three other types of receptor antibodies are described in this volume: experimentally induced polyclonal antibodies, human autoantibodies, and anti-anti-hormone (= anti-idiotype) antibodies. The latter reagents are capable of recognizing hormone or neurotransmitter receptors via 'molecular mimicry' and are discussed in detail by Strosberg and Schreiber in Chapter 2, and also by Ross (Chapter 5), Kohn *et al.* (Chapter 9) and Fuchs *et al.* (Chapter 8) (see also Sege and Peterson, 1978; Shechter *et al.*, 1982; Wassermann *et al.*, 1982; Homcy *et al.*, 1982; Cleveland *et al.*, 1983).

I should like to thank all the authors contributing to this volume for providing up to date and critical reviews of high quality. Most of them were submitted on time! My thanks are also due to Jackie Needham for her expert secretarial assistance, and Richard Stileman of Chapman and Hall Publishers for his untiring efforts and enthusiastic support of this and other volumes in the *Receptors and Recognition* series.

REFERENCES

Beisiegel, U., Schneider, W.J., Goldstein, J.L., Anderson, R.G.W. and Brown, M.S. (1981), *J. Biol. Chem.*, **256**, 11923–11931.

Friesen, H.G., Shiu, R.P.C., Elsholtz, H., Simpson, S. and Hughes, J. (1982), *Ciba Found. Symp.*, **90**, 263–278.

Galfre, G. and Milstein, C. (1981), *Methods Enzymol.*, **73B**, 13–46.

Goding, J.W. (1980), *J. Immunol. Methods*, **39**, 285–308.

Harford, J., Lowe, M., Tsunoo, H. and Ashwell, G. (1982) *J. Biol. Chem.*, **257**, 12685–12690.

Homcy, C.J., Rockson, S.C. and Haber, E. (1982), *J. Clin. Invest.*, **69**, 1147–1154.

Kita, T., Beisiegel, U., Goldstein, J.L., Schneider, W.J. and Brown, M.S. (1981), *J. Biol. Chem.*, **256**, 4701–4703.

McMichael, A.J. and Fabre, J.W. (eds) (1982) *Monoclonal Antibodies in Clinical Medicine*, Academic Press, London.

Schwartz, A.L., Marshak-Rothstein, A., Rup, D. and Lodish, H.F (1981), *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 3348–3352.

Sege, K. and Peterson, P.A. (1978), *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 2443–2447.

Shechter, Y., Maron, R., Elias, D. and Cohen, T.R. (1982), *Science*, **216**, 542–544.

Simpson, J.S.A., Hughes, J.P. and Friesen, H.G. (1983), *Endocrinology*, **112**, 2137.

Wassermann, N.H., Penn, H.S., Freimuth, P.I., Treptow, N., Wentzel, S., Cleveland, W.L. and Erlanger, B.F. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4810–4814.

Yelton, D.E. and Scharf, M.D. (1981) *Ann. Rev. Biochem.*, **50**, 657–680.

Contents

1 Introduction

MELVYN F. GREAVES

Monoclonal Antibodies to Receptors: Probes for Receptor Structure and Function
(*Receptors and Recognition*, Series B, Volume 17)
Edited by M. F. Greaves
Published in 1984 by Chapman and Hall, 11 New Fetter Lane, London EC4P 4EE

1.1 DIVERSITY OF APPLICATIONS OF ANTI-RECEPTOR ANTIBODIES

Successful applications of monoclonal antibodies to receptor biology are remarkably diverse (Table 1.1) and are perhaps best exemplified by the many elegant studies on nicotinic acetylcholine receptors which range from gene cloning and detailed protein subunit anatomy to the immunopathology of myasthenia gravis.

In many of these studies it is clear that monoclonal antibodies have had distinct advantages. Since these reagents can be selected for high affinity and bind to a single epitope, they permit a fine anatomical dissection of a receptor molecule and its subunits (see for example chapters by Ross, Fraser, Kohn *et al.*, Fuchs *et al.*, and also Sanchez-Madrid *et al.*, 1983; Tzartos *et al.*, 1981; Gullick and Lindstrom, 1983) and enable functional effects of antibodies to be linked to a particular site. The species specificity of selected monoclonal antibodies has also been important for allocating receptor genes to particular chromosomes by somatic cell genetics (references in Table 1.1; see also Goodfellow and Solomon, 1982).

1.2 SPECIFICITY OF ANTIBODIES TO RECEPTORS

It is important to appreciate that different regions or subunits of receptors are not equipotent immunologically; as far as a mouse immune response (for example) is concerned, certain areas of receptor will be 'immunodominant' (see Tzartos *et al.*, 1981). In addition, the physical form of the receptor material used for both immunization and hybridoma screening has a marked influence on the spectrum of antibody specificities and affinities obtained. Thus antibodies raised and selected against isolated receptors or their subunits may preferentially recognize intracytoplasmic domains of receptor unavailable on the cell surface (cf. Froehner, 1981) or subunit determinants which are cryptic or unavailable in the complete, native structure. Also, since isolation of receptors usually involves denaturation, antibodies raised against purified receptors may not recognize conformation-dependent regions or may only do so with low affinity (Tzartos *et al.*, 1981). As discussed by Fuchs *et al.* (Chapter 8), antibodies raised against denatured versus non-denatured receptors do show the anticipated specificity differences.

The hybridoma screening or selection procedure itself introduces a strong bias into the apparent repertoire of antibody specificities. Thus selective screening of monoclonal antibodies by inhibition of ligand binding may create the impression that many or most antibodies raised against receptors recognize the receptor's binding site for hormone, neurotransmitter, etc., whilst in fact this is almost certainly not the case. Firstly, although antibody binding may prevent interaction of receptor with its natural ligand (e.g. acetylcholine) or

Table 1.1 Some examples of applications of monoclonal anti-receptor antibodies

	Receptors (R)	Reference (example)
1. Affinity purification of receptors	Trf R and others	See Chapter 13 by Schneider; also Jacobs and Cuatrecasas (1981)
2. Biosynthesis, processing and membrane insertion of receptors	ACh R	Anderson and Blobel (1981) Fuchs *et al.* (Chapter 8)
	Insulin R	Hedo *et al.* (1983) Chapter 11 by Jacobs *et al.*
	IL-2 R	Chapter 3 by Leonard *et al.*
	Trf R	Schneider, C. *et al.* (1982) Chapter 10 by Trowbridge and Newman
3. Biochemical characterization, subunit anatomy and inter-relationships	AChR	Tzartos *et al.* (1981, 1982) Gullick and Lindstrom (1983) Chapter 8 by Fuchs *et al.*
	β_2 Adr R	Chapter 6 by Fraser
	T cell Ags	Chapter 7 by Terhorst
	LDL R	Schneider, W.J. *et al.* (1982)
	C3b R	Chapter 5 by Ross Springer *et al.* (1982)
	Steroid H R	Chapter 4 by Moncharmont and Parikh
	Asialo GP R	Schwartz *et al.* (1981) Harford *et al.* (1982)
4. Receptor localization *in situ*	Trf R	Gatter *et al.* (1983)
	Steroid H R	Chapter 4 by Moncharmont and Parikh
	ACh R	Swanson *et al.* (1983)
5. Identification of 'traffic' pathways for receptor–ligand internalization and recycling	Trf R	Hopkins and Trowbridge (1983) Enns *et al.* (1983)
	Asialo GP R	Geuze *et al.* (1983)
	LDL R	Brown *et al.* (1983)
	EGF R	Chapter 12 by Schlessinger *et al.* (See also Cuatrecasas and Roth, 1983 and Brown *et al.* (1983) for review)

6. Functional studies of receptor–response coupling	TSH R	Chapter 9 by Kohn *et al.*
	EGF R	Chapter 12 by Schlessinger *et al.*
	Trf R	Chapter 10 by Trowbridge and Newman
	IL 2 R	Chapter 3 by Leonard *et al.*
	Insulin R	Chapter 11 by Jacobs *et al.*
	T cell Ags	Chapter 7 by Terhorst See also Chapter 2 by Strosberg and Schreiber
7. Genetic mapping of receptors	EGF R	Waterfield *et al.* (1982)
	Trf R	Goodfellow *et al.* (1982)
8. Molecular cloning of receptors	ACh R	See Chapter 13 by Schneider
	Trf R	
9. Receptors, antibodies and disease pathology	TSH R	Chapter 9 by Kohn *et al.*
	ACh R	Chapter 8 by Fuchs *et al.* Tzartos *et al.* (1982)
	β_2 Adr R	Chapter 6 by Fraser; see also Chapter 2 by Strosberg and Schreiber for review.
	LDL R	Tolleshaug *et al.* (1982) Beisiegel *et al.* (1981)

TSH, thyroid-stimulating hormone/thyrotropin; ACh, acetylcholine; Trf, transferrin; IL-2, interleukin 2; β_2 Adr, adrenergic (receptor); T cell Ags, T lymphocyte, cell surface antigens; Steroid H, steroid hormone; EGF epidermal growth factor; Asialo GP, asialoglycoprotein; C3b, complement component(s) C3b (see Chapter 5 by Ross); LDL, low-density lipoprotein.

various agonists/antagonists (e.g. α-toxin) used as affinity probes for the binding site, the reverse is often not the case (see Fuchs *et al.*, Chapter 8). Assuming that the antibodies do not possess a much higher affinity for the receptor than the binding site specific probe (which seems very unlikely with, for example, the acetylcholine receptor), this observation suggests that immunoglobulins recognize epitopes close to but distinct from the receptor binding site(s) and inhibit by steric hindrance.

Secondly, there are theoretical reasons for anticipating a low or modest frequency of anti-binding site antibodies. Most of the receptors discussed in this volume have phylogenetically conserved combining site specificity; other, functionally less important, regions of the structure would be expected to have accumulated more sequence polymorphism and potential antigenicity. In