

ADVANCED IMMUNOCHEMISTRY

SECOND EDITION



ADVANCED IMMUNOCHEMISTRY

SECOND EDITION

Eugene D. Day, Ph.D.
Professor Emeritus of Immunology
Department of Microbiology and Immunology
Duke University Medical Center
Durham, North Carolina

 **WILEY-LISS**

A JOHN WILEY & SONS, INC., PUBLICATION
New York • Chichester • Brisbane • Toronto • Singapore

**Address all inquiries to the Publisher
Alan R. Liss, Inc., 41 East 11th Street, New York, NY 10003**

Copyright © 1990 Wiley-Liss, Inc.

Printed in United States of America

Under the conditions stated below the owner of copyright for this book hereby grants permission to users to make photocopy reproductions of any part or all of its contents for personal or internal organizational use, or for personal or internal use of specific clients. This consent is given on the condition that the copier pay the stated per-copy fee through the Copyright Clearance Center, Incorporated, 27 Congress Street, Salem, MA 01970, as listed in the most current issue of "Permissions to Photocopy" (Publisher's Fee List, distributed by CCC, Inc.), for copying beyond that permitted by sections 107 or 108 of the US Copyright Law. This consent does not extend to other kinds of copying, such as copying for general distribution, for advertising or promotional purposes, for creating new collective works, or for resale.

Library of Congress Cataloging-in-Publication Data

Day, Eugene D.
Advanced immunochemistry / Eugene D. Day.—2nd ed.
p. cm.
ISBN 0-471-56686-1—ISBN 0-471-56768-X (pbk.)
1. Immunochemistry. I. Title.
[DNLM: 1. Immunochemistry. QW 504 D273a]
QR183.6.D39 1990
616.07'9—dc20
DLC
for Library of Congress 89-70635
CIP

PREFACE

"Scientists whose work is prospering are wrapped up in it with such obsessive absorption that they want above all to be left to cultivate their gardens. They are not more than idly curious about what goes on in other people's gardens, and their closest approximation to neighborly behavior often amounts to little more than an inclination to borrow their neighbor's garden tools—especially the physicist's."

*—In My Neighbor's Garden
Sir Peter Medawar (1984)*

Let's linger a while at the fence overlooking the immunochemical garden and learn how we reached the highly cultivated stage of immunology in which we now find ourselves.

Talmage (1986) reminds us that "in the 30 years before 1948, the word 'lymphocyte' did not appear in the index of the **Journal of Immunology**". This merely punctuates the fact that Immunology has undergone its series of fundamental scientific revolutions in a very short period of time, so much so that a sizeable fraction of the living members of the American Association of Immunologists, for example, has witnessed the complete series.

Probably, a century from now, this whole period will be called an epoch by those philosophers of science who describe universally recognized scientific achievements in terms of paradigms (Kuhn, 1970). Horace Freeland Judson (1979) recalls talking with Max Delbrück about revolutions in science (they had just been discussing the Watson-Crick denouement), outlining for him how Kuhn might view things—"the epoch's ruling way of conceptualizing theories, so that the end of a scientific epoch is marked by the total breakdown of the paradigm and its replacement with another." Thus, some future philosopher of science might envision Immunology as taking a prominent place beside such other great paradigms as Darwinism, and Quantum Mechanics. Judson and Delbrück, agreeing "that 'paradigm,' as a term, was already almost thoroughly debauched as 'charisma'", questioned whether there were "only four or five revolutions in all of history of science big enough to meet the criteria". Judson quotes Delbrück as wondering, in fact, "whether there aren't hundreds. Except we don't know about them. Some few are vaunted. I don't know. I haven't read Kuhn's book."

If we are not careful, one such revolution that is certain to be overlooked in some future assessment of the Immunological Epoch (IE) is the immunochemical story. As the first of several chapters in the advancing saga of IE, it has already lost its romantic flavor in most circles in spite of its fundamental importance. Our attention has now turned to other episodes

and their unfolding stories which still keep us spellbound. And the final chapter of this great paradigm, still to be written, will no doubt be the one that will become recorded in philosophical history. It must be remembered, however, that Immunochemistry set the stage, defined the rules, and determined the nature and limits of specificity, cross-reactivity, complementarity, affinity, and heterogeneity, the five principles that permeate the whole of IE, even the world of that unwritten last chapter. Hopefully, the following pages will have captured enough of the essence of the first chapter of IE to make even the last chapter (as well as those in between) more meaningful. From more than 20,000 titles representing the body of immunochemistry I selected 7500 that appeared to have merit, retrieved the corresponding papers for critical examination, and found about 2200 that, in particular, encompassed much of the relevant material. Since my intention has always been to illustrate certain points, I have, of course, not been able to be encyclopedic in my coverage. Consequently, I have had to refrain from citing more than 2000 excellent and worthy papers in the final version, including a number from my own list of particular favorites, difficult though that has been. I do humbly apologize to my colleagues for any glaring omissions that have been made and truly hope they understand.

E.D.D.

Loudonville, N.Y.

March 31, 1989

References

- Judson, H.F. (1979). *The Eighth Day of Creation. The Makers of Revolution in Biology.* Simon and Schuster (New York, 686 pp.) p.61.
- Kuhn, T.S. (1970). *The Structure of Scientific Revolutions.* Univ. Chicago Press, Chicago, 2nd ed., 210 pp.
- Medawar, P.B. (1984). *The Limits of Science.* Harper and Row (New York, 108 pp.) p. 72.
- Talmage, D.W. (1986). The acceptance and rejection of immunological concepts, *Annu Rev. Immunol.* 4:1-11.

ACKNOWLEDGMENTS

There are four individuals in particular to whom I owe a debt of gratitude: my wife, Shirley, for her love and for her protective and faithful assistance in so many ways over so many years; my assistant at Duke University, Mr. Robert T. Holeman, for his professional bibliographic assistance, for his faithful transformation of my hand-written manuscript into an Apple IIe creation, and for his excellent management of my office at Duke University after I "retired" to my home in Loudonville, New York, to complete the manuscript; and my editors at Alan R. Liss, Dr. Brian Crawford and Mr. Tony Battle, who so efficiently managed the transformation of the Apple IIe product into its present form. I should also like to express my sincere thanks to the many authors and publishers, cited separately in text and references, for their generous and cooperative permission to include copyrighted material in these pages.

E.D.D.

CONTENTS

Preface	xxi
Eugene D. Day	xxi
Acknowledgments	xxiii

Part One

Structure of Antibodies

1	The Light Chains of Immunoglobulins	3
1.1.	Bence-Jones proteins	4
1.2.	Immunoglobulin light chains and Bence-Jones proteins	6
1.3.	Amino acid sequences of human kappa and lambda light chains	7
1.4.	Subgroups of the variable regions of human kappa and lambda chains	13
1.5.	The constant regions of human kappa and lambda chains: genetic polymorphism and the development of allotypes and isotypes	15
A.	The Km allotype	15
B.	Lambda chain isotypes	17
1.6.	Primary amino acid sequences of light chains from various species	18
A.	Sequence comparisons among mouse, rat, and rabbit kappa chains	18
B.	Mouse lambda chains	22
C.	Allelism in the light chains of rabbit immunoglobulins	23
D.	Hypervariability	25
E.	Light chains among the species	26
1.7.	Light chain gene organization	28
A.	Proof of a hypothesis	28
B.	The mechanism of V-C joining	29
C.	The number of V-region genes	33
D.	Allelic and isotypic exclusion	34
E.	The CDR	36
F.	The leader sequence	38

8.	Stability, flexibility, and three-dimensional structure of the light chain	38
A.	The importance of sulfur	38
B.	Hydrophobicity	39
C.	Glycines and the CDR	40
D.	Three-dimensional structures	41
E.	Dimerization	43
	References	44

2	The Heavy Chains of Immunoglobulins	53
2.1.	The organization of heavy chain genes	54
2.2.	The evolution of heavy chain constant region sequences	55
2.3.	Gamma-chain constant region sequences	70
A.	Allotypy	70
B.	Constant region sequences	75
2.4.	The mu-chain region sequences	75
A.	Allotypy	75
B.	Membrane vs. secreted forms	78
2.5.	The membrane vs. secreted forms of other immunoglobulin chains	79
2.6.	The delta-chain constant region sequences	79
A.	Transcriptional regulation of mu- and delta- gene expression	79
2.7.	The epsilon-chain constant region sequences	80
A.	A truncated pseudogene, the human C _e 2 gene	80
B.	Production of epsilon chains by genetic engineering	80
C.	Evolutionary considerations	80
2.8.	The alpha-chain constant region sequences	81
A.	Secreted vs. membrane-associated forms	81
B.	Isotypes of human alpha chains	81
C.	Allotypy	81
2.9.	Natural hybrids	84
2.10.	Deletions	85
2.11.	The variable region of heavy chains	86
A.	V _H DJ _H rearrangements	86
B.	J _H regions	86
C.	D-region sequences	86
D.	Variable region families	88
E.	V _H subgroups and families	90
F.	Phylogenetic aspects	94
G.	V _H region allotypes in rabbit heavy chains	94
1.	The nominal allotype α	94
2.	The α -negative allotype	98
3.	Latent α allotypes	98

H. V_{H} -region allotypes in the human	98
I. V_{H} -precursor amino acid sequences	98
References	99

[3] The Secondary, Tertiary, and Quaternary Structures of Assembled Immunoglobulins	107
3.1. The early model of IgG	108
3.2. Current model of IgG—disulfide bonds	112
3.3. Conformational aspects of IgG and a more intimate view of its structure	116
3.4. Light chain contribution to whole molecule conformation	122
3.5. The anti-parallel β -pleated sheet—the “predominant structural feature” of an immunoglobulin subdomain	124
3.6. The separation of the two $C_{H}2$ domains in an assembled immunoglobulin	124
3.7. The $C_{H}3$ dimer, pFc'	125
3.8. Segmental flexibility	125
3.9. The flexible Noelken-Tanford model revisited	129
3.10. Fragments	130
3.11. The structure of Fab	130
3.12. The structure of IgM	132
A. Disulfide bonds	132
B. The Feinstein model	133
C. Electron microscopy and an early view	134
D. The circulating IgM monomer	134
E. J-chain structure	136
1. Biosynthesis	136
2. Primary, secondary, tertiary structure	137
3. Comparative studies	138
F. The fragments of IgM	138
G. Segmental flexibility	138
H. Heavy chain disease (HCD) proteins among IgM molecules	139
I. Oligosaccharide units	140
3.13. The structure of IgD, the mystery Ig	140
A. The case of the missing $C_{H}2$ domain of mouse IgD	140
B. The hinge region	140
C. Structural heterogeneity	141
D. Membrane vs. secreted forms and search for a function	141
E. Fragments and conformation	141
3.14. The structure of IgA	142
A. Secretory IgA	142
B. Secretory component structure	143
C. Role of the J chain	147
D. Fragments of IgA1	148

Contents

E.	Alpha-chain disease	148
F.	Comparative studies	150
G.	Carbohydrate	150
H.	A note on the membrane form	150
3.15.	The structure of IgE	151
3.16.	Biosynthesis and bioassembly	154
	A. Early studies	154
	B. Variants	155
	C. Models for covalent assembly	156
	D. Cell-free synthesis	157
	E. The modern view	157
	F. The role of carbohydrate	158
	G. Chimeric immunoglobulins	158
	H. Deletion mutants	160
3.17.	Distribution of immunoglobulins among the fluids and tissues of the body	161
3.18.	Phylogenetic considerations	163
	A. The hagfish, the most primitive of the vertebrates	163
	B. Phylogenetic origins of immunoglobulin structure	164
	C. Evolution of the core of the antibody domain	168
	D. The basic immunoglobulin fold	168
	References	170
4	The Antibody Binding Site	183
4.1.	Antibody specificity and primary structure	185
4.2.	Defeat of the Instructionists	186
4.3.	Specific interaction of V_H and V_L regions	188
4.4.	Preferential reassociations	189
4.5.	Affinity labeling of antibody binding sites	190
	A. The enzyme analogy	190
	B. The affinity labeling of antibodies, early studies	191
	C. The major fallacy of "protection" experiments	191
	D. Tyrosine in antibody binding sites as determined by affinity labeling	193
	E. Lysine in antibody binding sites as determined by affinity labeling	193
	F. Isoelectric focusing of affinity-labeled anti-polysaccharide antibodies	194
	G. Photoaffinity labels	194
4.6.	Spin-labeled probes in the study of the antibody combining site	196
4.7.	Nuclear magnetic resonance (NMR) spectra, useful tools in the refinement of combining site architecture	199

4.8.	Fluorescent probes of antibody active sites	203
4.9.	Extrinsic cotton effects	207
4.10.	Conformational changes in binding sites due to ligand binding	208
4.11.	Antigenic accommodation as a measure of combining site size	213
	A. Lower size limit	213
	B. Upper limit	215
	C. The nature and sizes of the combining sites of homogeneous myeloma proteins	216
	D. The nature and sizes of the combining sites of homogeneous monoclonal antibodies	218
	E. Subsites and multispecificity	218
	F. Multispecificity	219
4.12.	The three-dimensional structure of the variable region	220
	A. The primary amino acid sequences	220
	B. Three-dimensional structures of antibody binding sites	223
	1. Immunoglobulin molecule KOL	223
	2. McPC 603, a mouse myeloma protein with phosphorylcholine (PC)-binding activity	223
	3. Mouse myeloma protein J539, a galactan-binding immunoglobulin	224
	4. Human myeloma protein NEW	224
	5. Monoclonal antibody D1.3	224
	6. Monoclonal Gloop antibodies	226
	7. Monoclonal anti-lysozyme antibody Hy HEL-10	228
	8. Myeloma protein M603	228
	9. The <i>M_g</i> light-chain dimer	228
	10. The MOPC 315 light chain dimer vs. Fv	230
4.13.	Idiotype	231
	A. Discovery and definition: early explorations	231
	B. Cross-reactive idiotypes	234
	1. The CRI of anti-Ars	234
	2. The idiotypic cross-reactivity of antibodies against the phosphorylcholine (PC)-binding region of mouse IgA TEPC 15	237
	3. J558 and M104E	238
	4. The idiotopes of anti-streptococcal group A carbohydrate (anti-GAC)	239
	5. Rabbit antibodies to group C carbohydrate (C-CHO)	239
	6. The GAT system	240
	7. The NP ^b idotype	240
	8. MOPC 460 idiotopes	240
	9. V _k 21 light chain idiotypes	241
	10. Different specificities, similar idiotypes	241
	11. Two different idiotypes for anti-lysozyme antibodies	241
	12. Xenopian idiotypes	242
	13. Shared idiotypes	242
	14. Synthetic idiotypes	242

xii Contents

15. Idiotope vaccines, the internal image, and the idiotypic network	243
References	244

[5] Effector Functions of Immunoglobulins 257

5.1. Immunoglobulins as B-cell receptors	258
5.2. Specific cell binding	258
5.3. Complement	258
A. Introduction	258
B. C1q	259
C. The C1 ₁ C1 ₂ tetramer	261
D. The C1q receptor site on IgG	261
E. The C1q binding site of IgM	263
F. C1q binding to non-immune rabbit IgG	263
5.4. Immune clearance	263
5.5. The IgE receptor	265
A. RBL cells and the IgE receptor	265
B. The cross-linking of IgE molecules on mast cells and RBL cells ..	265
C. The locale of receptor binding sites on IgE molecules	266
D. Segmental flexibility	268
5.6. Transport	269
A. Maternofetal transmission of IgG	269
B. Transepithelial transport of IgA	271
C. Transport across the intestinal mucosa	272
5.7. The Fc receptors of other cells	272
A. Monocytes	272
B. Fc receptors on K cells	272
5.8. An isotopic network based on Fc binding	273
5.9. Conclusion	273
References	274

Part Two

Reactions of Antibodies

[6] Specificity and Complementarity 281

6.1. Haptenic complementarity	281
6.2. Full epitopic complementarity	285
6.3. The limits placed upon effective complementarity	286
6.4. Specificity and cross-reactivity	287

6.5.	Structural specificity, haptic and epitopic	288
6.6.	Distributive, the second kind of specificity/cross-reactivity	290
6.7.	Discriminatory specificity/cross-reactivity	291
	References	292
7	Affinity	295
7.1.	Introduction	296
7.2.	Rigorous derivation of ideal affinity equations	296
7.3.	Various special cases of $[rlc = nK_{12} - srK_{12}]$	298
	A. Special case of univalent antigen or hapten	298
	B. Special case in far antigen excess when antigen is not univalent	299
	C. Special case of equivalence	300
7.4.	Various special cases of $[f/d = sK_{12} - \eta fK_{12}]$	300
	A. Special case of equilibrium filtration near equivalence with bivalent antibody	301
	B. Special case of equilibrium filtration in far bivalent antibody excess	301
7.5.	Extent of the reaction—the Karush equation	302
7.6.	Langmuir form of affinity equation	304
7.7.	Heterogeneity constants	304
7.8.	Interrelationships	305
7.9.	Functional affinity, multivalency, and monogamous bivalent binding	306
7.10.	The Steward-Petty approximation	307
7.11.	Affinity heterogeneity	310
	A. Early studies by equilibrium dialysis	310
	B. Heterogeneity constant	311
	C. Affinity heterogeneity, a normal state of the polyclonal immune response	314
	D. Quantum-sensitive probes in the measurement of affinity and affinity heterogeneity	317
	E. DNS and ANS haptens	318
	F. Arsonate hapten with single and double charges	320
	G. Heterogeneity in inbred mice	320
	H. Effect of carrier residues on heterogeneity— lysine vs. guanosine	320
	I. Heterogeneity and heavy chains	321
	J. Affinity, heterogeneity, and the immune responses	321
	K. The Farr technique for determining binding parameters	324
	L. Heterogeneity vs. homogeneity	327
7.12.	Kinetics of hapten—antibody interaction	327

A. Introduction	327
B. Temperature-jump relaxation	328
C. Stopped flow fluorescence polarization	330
D. A simplified experimental approach to the determination of antibody-hapten association kinetics	335
7.13. Dissociation kinetics	337
7.14. Non-Sipsian affinity heterogeneity	339
A. Skewed affinity heterogeneity	339
B. Discontinuous affinity heterogeneity	341
C. The reverse Rosenthal method	341
D. Distribution of affinities after fractionation	345
E. Patterns of affinity	345
References	346

8 The Drive Toward Homogeneity and the Advent of Monoclonal Antibodies

8.1. Homogeneity and clonal selection—the Klinman experiment	352
8.2. Homogeneous binding of nitrophenyl ligands by myeloma proteins	355
A. The nitrophenyl group	355
B. The anti-phosphoryl choline group of homogeneous myeloma proteins	358
C. Myelomas with reactivity for dextrans, levans, galactans, and other polysaccharides	359
D. Human myeloma immunoglobulins	360
8.3. The continued search for antibodies with molecular uniformity and restricted heterogeneity	361
A. Homogeneous antibodies to streptococcal, pneumococcal, and micrococcal ligands	361
B. Restricted heterogeneity in the antibody responses to other ligands	368
8.4. The hybridoma revolution	370
A. Introduction	370
B. Purity of monoclonal antibodies (MAb)	371
C. Screening hybridoma cell products for ligand specificity	372
D. Affinities of monoclonal antibodies	373
E. The determination of intrinsic affinity of monoclonal antibodies	375
1. Ligand heterogeneity	375
2. The use of an indirect ELISA to obtain intrinsic affinity constants	377
3. Monoclonal antibodies in equilibrium dialysis with protein ligands	379
4. Hapten-enhanced antibody binding	379
F. Mapping of antigenic regions with MAb	380
G. MAb specific for classes of proteins	385

11. Families of MAb with shared idiotypes exemplified by anti-PC	387
I. Idiotype cross-reactivity, disclosed through MAb	388
J. Diversification of MAb	388
K. MAb as enzymes and the emergence of "abzymes"	389
References	389
9 Immune Complexes	397
9.1. Introduction	398
9.2. Quantitative precipitation of pneumococcal polysaccharides	401
9.3. Precipitation and solubilization in antigen-excess regions	409
9.4. The Goldberg equation	411
9.5. Ultracentrifugation of immune complexes	416
9.6. Circular antibody-antigen complexes	419
9.7. Precipitin reactions of MAb with antigens containing repeating epitopes	421
9.8. Non-precipitation	423
A. Monogamous bivalent binding of equine IgG(T)	423
1. Historical note	423
2. The experiments of Klinman and Karush	424
3. The experiments of Archer and Krakauer	426
B. Other types of non-precipitating immunoglobulins	426
1. Porcine antibodies	426
2. Mouse antibodies	427
3. Guinea pig antibodies	427
4. Rat antibodies	427
5. Rabbit antibodies	427
9.9. Antigenic valence	428
A. Introduction	428
B. The Heidelberger plot	429
9.10. Antibody multivalence	432
A. Virus neutralization with antibody: role of antibody bivalency	432
1. Introduction	432
2. Phage neutralization with antibody	437
3. Neutralization of modified phage by antibody	438
B. Restrictions on monogamous bivalent binding	445
9.11. The salting-out of immune complexes	447
A. Introduction	447
B. Reequilibration	447
9.12. Radioimmunoassay binding curves	449
9.13. Solid-phase immunoassay and affinity	451
A. Introduction	451
B. Cooperativity in polyclonal antibody reactions with solid-phase systems	452

Contents

C.	Equilibrium constants of MAb by solid-phase immunoassays	454
D.	Bivalent antibody binding with monovalent ligand on a cell surface	455
E.	The kinetics and thermodynamics of antigen-antibody reactions in heterogeneous reaction phases	456
F.	The analytical affinity chromatography assay	457
9.14.	Solubilization of immune complexes	460
9.15.	Lipid antigens, membranes, and spin labels	460
	References	461

10	The Immunochemistry of Viruses	469
10.1.	Tobacco mosaic virus (TMV)	470
A.	Introduction	470
B.	Early antigenic studies	470
C.	Homogeneity and antigenic valence	473
D.	Multivalence of the TMV coat protein	474
1.	The two kinds of antigenic valency	474
2.	TMVP is not effectively univalent but neither is it only pentavalent	475
3.	Heteroclitic (heterospecific) antibodies	475
4.	Segmental mobility	476
10.2.	Turnip yellow mosaic virus (TYMV)	476
10.3.	Poliovirus	478
10.4.	Human rhinoviruses	480
10.5.	Reovirus	481
10.6.	Foot-and-mouth disease virus (FMDV)	482
10.7.	Cooperative effects among anti-viral antibodies	485
10.8.	Retroviruses	486
10.9.	The human hepatitis B virus (HBV)	487
10.10.	The antigenic structure of rabies virus glycoprotein	492
10.11.	Influenza viruses	494
A.	Structure and antigenic variation	494
B.	A detailed look at an updated HA1 map	498
C.	"The structure of an antigenic determinant in a protein"	498
D.	Another synthetic determinant and the problem of conformation	500
E.	Conformational changes accompanying fusion	503
F.	Antigenic changes correlated with changes in specific receptor binding	503
G.	Neuraminidase	504
H.	Influenza viruses types B and C	505

I.	Antigenic variation of internal influenza virus A proteins	505
J.	Interrelationships in HA among mammalian and avian influenza viruses	506
K.	Equilibrium filtration, original antigenic sin (OAS), and the question of antigenic valence	506
L.	Monogamous bivalent binding	511
10.12.	The neutralization of MS2 coliphage—"the first reported evidence that a synthetic peptide could be utilized for eliciting a neutralizing anti-viral response"	513
	References	514

[11] The Immunochemistry of Some Non-Viral Multiepitopic Antigens 521

11.1.	Serum albumins	523
A.	Introduction	523
B.	Identity of the F1 fragment	524
C.	Mapping of the epitopes of HSA and BSA	524
D.	Affinities	527
11.2.	Myoglobin	527
A.	Structure	527
B.	An antibody-induced conformational change	530
C.	Sequential determinants	530
D.	Topographic determinants	531
E.	Peptide fragment determinants	532
F.	Continuous antigenic determinants revisited	532
11.3.	Hemoglobin	537
A.	Quaternary structure and determinants	537
B.	Sickle hemoglobin	539
C.	Valence	539
D.	Determinant mapping, preliminary studies	541
1.	Amino acid mutants	541
2.	The synthetic peptide approach	541
11.4.	Lysozyme	543
A.	Structure	543
B.	The "loop" peptide	544
1.	Initial discovery	544
2.	Binding parameters	545
3.	The effect of changes in specific amino acid residues of the loop upon antigenicity	546
4.	Ploop I-II	547
5.	Gloop 1-5	548
6.	Atassi and the loop	548