Immunoglobulin Genes and B Cell Differentiation

Editors:
Jack R. Battisto/Katherine L. Knight

Developments in Immunology Volume 12

IMMUNOGLOBULIN GENES AND B CELL DIFFERENTIATION

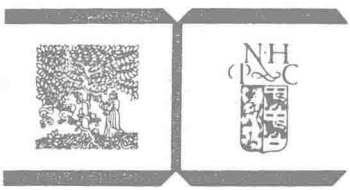
Proceedings of the 8th Annual Mid-West Autumn Immunology Conference, Detroit, Michigan, U.S.A., November 4–6, 1979

Editors:

JACK R. BATTISTO, Ph.D.
Scientific Director, Department of Immunology, Cleveland Clinic Foundation

and

KATHERINE L. KNIGHT, Ph.D.
Professor of Microbiology, University of Illinois Medical Center



ELSEVIER/NORTH-HOLLAND
NEW YORK • AMSTERDAM • OXFORD

Published by:

Elsevier North Holland, Inc.
52 Vanderbilt Avenue, New York, New York 10017

Sole distribution outside of the United States and Canada:

Elsevier/North-Holland Biomedical Press
335 Jan van Galenstraat, P.O. Box 211
Amsterdam, The Netherlands

Library of Congress Cataloging in Publication Data

Mid-west Autumn Immunology Conference, 8th, Detroit, 1979.
Immunoglobulin genes and B cell differentiation.
(Developments in immunology; 12 ISSN 0163-5921)

Bibliography: p.
Includes index.
1. Immunology—Congresses. 2. Immunoglobulins—Congresses.
3. B cells—Congresses. 4. Cell differentiation—Congresses.
5. Immunogenetics—Congresses. I. Battisto, Jack Richard, 1922-
II. Knight, Katherine L. III. Title. IV. Series. [DNLM:
1. Immunoglobulins—Congresses. 2. Genes, Immune response—
Congresses. 3. B-Lymphocytes—Immunology—Congresses. 4. Cell
differentiation—Congresses. W1 DE997WM v. 12 / QW 601 M627 1979i]
QR180.3.M52 1979 616.07'9 80-25347
ISBN 0-444-00580-3
(0-444-80028-X-Series)

Manufactured in the United States of America

IMMUNOGLOBULIN GENES AND B CELL DIFFERENTIATION

DEVELOPMENTS IN IMMUNOLOGY

Volume 1—Genetic Control of Autoimmune Disease, edited by Noel R. Rose, Pierluigi E. Bigazzi and Noel L. Warner, 1978

Volume 2—Immunology of Bacterial Polysaccharides, edited by Jon A. Rudbach and Phillip J. Baker, 1979

Volume 3—B Lymphocytes in the Immune Response, edited by Max Cooper, Donald E. Mosier, Irwin Scher and Ellen S. Vitetta, 1979

Volume 4—Immunologic Tolerance and Macrophage Function, edited by Peter Baram, Jack R. Battisto and Carl W. Pierce, 1979

Volume 5—Aging and Immunity, edited by S.K. Singhal, N.R. Sinclair and C.R. Stiller, 1979

Volume 6—Immunobiology and Immunotherapy of Cancer, edited by William D. Terry and Yuichi Yamamura, 1979

Volume 7—Virus-Lymphocyte Interactions: Implications for Disease, edited by Max Rowland Proffitt, 1979

Volume 8—Development and Differentiation of Vertebrate Lymphocytes, edited by John D. Horton, 1980

Volume 9—Immunological Aspects of Infertility and Fertility Regulation, edited by Dharam S. Dhindsa and Gebhard F. B. Schumacher, 1980

Volume 10—Phylogeny of Immunological Memory, edited by Margaret J. Manning, 1980

Volume 11—Infections in the Immunocrompromised Host—Pathogenesis, Prevention and Therapy, edited by Jan Verhoef, Phillip K. Peterson and Paul G. Quie, 1980

Volume 12—Immunoglobulin Genes and B Cell Differentiation, edited by Jack R. Battisto and Katherine L. Knight, 1980

Preface

The Mid-West Autumn Immunology Conference, which is eight years young, has focussed its attention for this year's session upon humoral immunity. Of the two symposia presented, one centered upon how antibody diversity is controlled by immunoglobulin genes and the other dealt with B cell differentiation-activation. Bringing together on one program several experts in each of these topics had the stimulating effect of generating discussion not only on the separate subjects but on the areas of overlap, as well. Of particular interest were the questions of how precursor cells go through the various stages of differentiation to become antibody synthesizing plasma cells, when B cells acquire membrane markers and receptors, how the B cell immunoglobulin surface receptors of antigen function to trigger messages for the cell, when as well as how clonal diversity is brought about, and how the genes that control antibody synthesis are rearranged and RNA is spliced so as to control differences seen in the constant as well as variable regions of the peptide chains that comprise the immunoglobulin molecules.

General discussions at the end of each symposium presentation and at the conclusion of each symposium were designed to foster interaction between the audience and among the symposia speakers. These discussions were taped and appropriately edited versions appear following each speaker's contribution.

The Mid-West Autumn Immunology Conference is also designed to have a number of workshops each of which is presided over by one or two moderators. They are designed to permit participating investigators who have submitted abstracts, to discuss their research interests and problems in small groups. This year the first hour of each workshop session was devoted to a poster session so that participants had the opportunity to examine data more critically and in greater detail. The following two hours were used for short oral presentations of the material appearing in the poster sessions. We have found this format promotes lively discussion by the moderator(s),

symposium speakers, and workshop participants. The contents of these workshops have been summarized by the moderator who, depending upon their own inclinations have preceeded the synopses with overviews of the particular areas of research. In this way some moderators have attempted to weave the short individual contributions into the larger fabric of existing knowledge.

Thus, with the exception of the stimulating verbal exchanges that occurred at the Mixer and at the Dinner, the proceedings of the entire Eighth Annual Mid-West Autumn Immunology Conference are contained in this text.

The Editors

Council Members

Mid-West Autumn Immunology Conference
1979

J. R. Battisto, Chairperson
Cleveland Clinic Foundation

W. E. Bullock, Jr.
University of Kentucky

J. E. Butler
University of Iowa

J. F. Clafin
University of Michigan

C. S. David
Mayo Medical School

J. W. Dyminski
Childrens Hospital,
Cincinnati

T. Huard
Postdoctoral Representative
University of Michigan

Y. M. Kong, Treasurer
Wayne State University

H. C. Miller
Michigan State University

H. B. Mullen, Workshop Coordinator
University of Missouri

C. W. Pierce
Washington University

L. S. Rodkey
Kansas State University

J. R. Schmidtke
University of Minnesota

T. Schindler
Graduate Student Representative
University of Illinois

D. Segre
University of Illinois

R. H. Swanborg, Secretary
Wayne State University

J. H. Wallace
University of Louisville

T. G. Wegmann
University of Alberta

Participants

Jerry A. Bash
Georgetown University

Jack R. Battisto
Cleveland Clinic Foundation

Jane Berkelhammer
University of Missouri

Ward E. Bullock, Jr.
University of Kentucky

John Butler
University of Iowa

Max D. Cooper
University of Alabama

David Crouse
University of Nebraska

Bert Del Villano
Cleveland Clinic Foundation

Thomas Feldbush
University of Iowa

James Finke
Cleveland Clinic Foundation

W. Carey Hanly
University of Illinois

Leroy Hood
California Institute of Technology

Norman Klinman
Scripps Clinic and Research

Katherine L. Knight
University of Illinois

Randall Krakauer
Cleveland Clinic Foundation

Stephen P. Lerman
Wayne State University

John Niederhuber
University of Michigan

Dennis Osmond
McGill University

Nicholas Ponzio
Northwestern University

Diego Segre
University of Illinois

Jonathan G. Seidman
National Institutes of Health

Roy S. Sundick
Wayne State University

Ellen S. Vitetta
University of Texas

Randolph Wall
University of California at Los Angeles

Thomas Wegmann
University of Alberta

Acknowledgments

We gratefully acknowledge the generous support of:

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
FOGARTY INTERNATIONAL CENTER
ABBOTT LABORATORIES
DIFCO LABORATORIES

We sincerely thank the sustaining sponsors:

ELI LILLY AND COMPANY
MERCK & CO., INC.
THE UPJOHN COMPANY

We also thank the contributing sponsors:

BECKMAN INSTRUMENTS, INC.
MICROBIOLOGICAL ASSOCIATES

Contents

I

Immunoglobulin Genes

Battisto/Knight, eds. Immunoglobulin Genes and B Cell Differentiation

ORGANIZATION OF IMMUNOGLOBULIN GENES - INTRODUCTORY REMARKS

KATHERINE L. KNIGHT
Department of Microbiology and Immunology, University of Illinois at the Medical Center, Chicago, Illinois 60680

The genetic origin of antibody diversity has intrigued immunologists and geneticists for many years. Amino acid sequence studies of Bence Jones proteins identified variable (V) and constant (C) regions of immunoglobulin (Ig) light chains and in 1965, Dreyer and Bennett[1] proposed that these variable and constant regions were encoded by separate genes. Strong support for this concept came from continued amino acid sequence studies on mouse and human κ-chains whereby a single constant region sequence was found associated with any one of multiple V region sequences. Likewise, in heavy chains, individual constant regions are associated with any of several V region sequences. In addition, individual V_H regions can be associated with any of the five heavy chain constant regions, C_γ, C_μ, C_α, C_δ, or C_ε. For example, allotypes of the variable region of rabbit heavy chains could be found on all classes of Ig molecules.[2,3] The simplest explanation for this observation is that the V_H gene can associate with genes for C_γ, C_μ, C_α, C_δ and C_ε. Additional support for the two gene-one polypeptide chain hypothesis came from studies on IgG and IgM monoclonal proteins isolated from one patient;[4,5] idiotypic and amino acid sequence analyses revealed that the V regions of these two molecules were identical whereas the C region represented different Ig classes. Again, the simplest explanation is that V and C regions are encoded by separate genes and that one V_H gene can be associated with both C_γ and C_μ.

Formal proof for the two gene-one polypeptide chain hypothesis was not obtained until 1976 when Tonegawa and his collaborators began direct analysis of the DNA. Initially, they showed that a probe for both the V and C regions

of mouse kappa chain (intact kappa chain mRNA) hybridized to two restriction fragments of mouse DNA, whereas a probe for the C region of the kappa chain (the 3'-end half section of the mRNA) hybridized to only one of these two DNA fragments.[6] Thus, the information for V and C regions appeared to be encoded in different DNA fragments. Subsequent studies on the genes coding for mouse lambda chains confirmed and extended these studies.

Embryonic DNA and DNA from a lambda chain plasmacytoma were cleaved by endonucleases and were subjected to agarose gel electrophoresis.[7] The fragments which hybridized to lambda chain mRNA were cloned in a lambda phage vector and the cloned DNA was subjected to R-loop analysis or to nucleotide sequence analysis. By R-loop mapping, the V and C genes were shown to be on different DNA fragments in embryonic DNA and in myeloma DNA, V and C were separated by an intervening sequence of 1250 base pairs.[7] Thus, the V and C regions of lambda chains were also encoded by separate gene segments. Since the V_λ and C_λ genes were much closer together in the myeloma DNA (approximately 1250 base pairs apart) than in the embryonic DNA (the distance between V and C is still unknown) a gene rearrangement must have occurred during differentiation to position the V and C genes closer together, albeit not contiguous. Thus, the Dreyer and Bennett hypothesis of separate genes for V and C had been confirmed.

The excitement over the Ig genes continued. Nucleotide sequence studies showed that in embryonic DNA the codons for the N-terminal 96 amino acids of V_λ were contiguous[8] but the codons for the C-terminal[13] residues of V_λ formed a separate gene segment, designated J_λ; The J_λ gene segment was found between the V_λ and C_λ gene segments, approximately 1250 base pairs to the 5' side of C gene.[9] In lambda chain myeloma DNA, the V_λ and J_λ gene segments were contiguous; thus, the somatic rearrangement which occurred during differentiation resulted in deletion of the intervening sequence between the V and J gene segments. The VJ-C intervening sequence of 1250 base pairs in the myeloma DNA

is found in the primary nuclear RNA transcript.[10] This VJ-C intervening sequence is deleted during RNA splicing and the final product is mRNA. The precise nature of the nuclear RNA and the mechanism of RNA splicing are under investigation in Dr. R. Wall's laboratory and will be discussed in detail in his presentation.

The organization of kappa chain genes has been extensively studied by Dr. P. Leder and his collaborators and the essential aspects of the gene organization are similar to those of the lambda chain genes.[11] The variable regions of mouse kappa chains are considerably more heterogeneous than those of lambda chains and examination of this system has allowed an estimate of the number of V_K genes in the germ line.[12] These studies are obviously important to understanding the genetic origin of antibody diversity and Dr. J. Seidman will describe the progress made in this area.

Studies on proteins obtained from patients with heavy chain disease have been of particular interest. Structural analyses of heavy chains isolated from these patients as well as heavy chains of some myeloma proteins have shown non-random deletions.[13,14] Many of the heavy chain mutants have the entire C_H1 domain deleted plus a large portion of the V domain; several other mutants have only the hinge region deleted. In variants where the C_H1 domain was deleted, the deletion usually ended at position 216, the beginning of the hinge region. These observations prompted the suggestion that the constant region of heavy chains may be encoded by more than one gene, one for C_H1, one for the hinge and at least one for the Fc portion of the heavy chain.[13] Thus, the possibility arose that heavy chains may be encoded by at least four genes. Recent studies of embryonic and myeloma DNA have confirmed that indeed, heavy chains are encoded by multiple gene segments, V_H, J, hinge and one for each C_H domain. Studies of the gene organization of heavy chains will be discussed by Dr. L. Hood.