Immunopathology

IIIrd International Symposium

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Edited by

Prof. Dr. Pierre Grabar, Paris

Prof. Dr. Peter A. Miescher, New York

IMMUNOPATHOLOGY

IIIRD INTERNATIONAL SYMPOSIUM 1963

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PREFACE

Immunopathology, i.e. the study of immune phenomena in disease, has attracted growing interest. The number of investigators devoted to this field is increasing at a great rate, and correspondingly, the number of contributions has been multiplying in recent years. This development is also reflected in the symposia on immunopathology. The first symposium was devoted to a variety of problems involving immunologic mechanisms. Investigators with broad and diverse interests discussed their mutual problems and points of view. For the second symposium it was found more desirable to limit the discussion to the problem of mechanisms of tissue damage produced by immune reactions, a field of immunopathology which has received special attention from many investigators. Only 15 months later, the third symposium took place. The fact that it was possible and desirable to hold another meeting after such a short interval without undue repetition gives evidence for the rapidity of development of interest in immunopathology.

The topics of the Third Symposium on Immunopathology were selected in order to cover some of the most active new developments in immunopathology. Much progress has been achieved in the understanding of the structure of immune globulins; with the characterization of the various structural subunits of the immune globulins, exact genetic studies have become possible, opening a new field of genetics of immune globulins.

The second and third topics dealt with the problem of antibody formation and the factors that influence it. This problem not only has broad biologic interest but also may have direct clinical applications.

In the fourth session, mechanisms of immunogenic kidney disease were discussed. A number of differing experimental immune mechanisms are known to produce damage to the kidney. However, in immunopathology of the human kidney, much work has still to be done in order to understand the differing pathogenic pathways involved in the various kidney disorders.

The last chapter deals with mechanisms of hematologic damage produced by immunological reactions. Formed elements of the blood are easily available for investigation, and thus lend themselves for the study of the mechanisms of cell damage produced by immune reactions. Special attention was given to the action of antigen-antibody complexes.

The Third Symposium on Immunopathology was very generously sponsored by the National Foundation and the Atomic Energy Commission. We wish to gratefully acknowledge their financial support and their understanding cooperation.

As in the previous proceedings, the pertinent discussions have been edited by a number of participants. Repetitions were eliminated whenever possible, and the sequence of the discussion was arranged in order to facilitate their reading by non-participants. We are especially indebted to Drs. B. Benacerraf, Charles G.

Cochrane, E. Franklin, and H. Müller-Eberhard for their collaboration in editing the discussions. We are grateful to all participants for their contributions and help in the organization of the symposium and in the publication of the proceedings.

In the preparation of this symposium and in its actual organization, Dr. G. McMahon acted again as an efficient secretary general. We gratefully acknowledge his invaluable help.

Dr. h. c. Christian Overstolz and Dr. H. G. Oeri from Schwabe & Co. spared neither trouble nor expense in the publication of the present volume. We owe our thanks to them. The distribution of the book in the United States of America has kindly been undertaken by the publishers Grune & Stratton, Inc., New York and London.

Paris and New York, September 1963 *Pierre Grabar* *Peter A. Miescher*

WELCOME

There is no doubt that immunopathology, in an environment of proper and integrated disciplines, is one of the most, if not the most, productive health sciences in our era. At meetings such as these, with immunopathology as the fundamental and common interest for a selected few who do so much, there is no question as to accomplishment. Accordingly, our institution is proud to be a participant in the proceedings of the Third International Symposium on Immunopathology.

EDMUND L. KEENEY, M.D.
Medical Director
Scripps Clinic and Research Foundation

K. Frank Austen, M.D., Department of Microbiology, The Johns Hopkins University School of Medicine, Baltimore 5, Maryland

Baruj Benacerraf, M.D., Professor of Pathology, Department of Pathology, New York University School of Medicine, 550 First Avenue, New York 16, New York

Samuel C. Bukantz, M.D., Medical and Research Director, Children's Asthmatic Research Institute of Health, 3447 West 19th Avenue, Denver 4, Colorado

Pierre Burtin, M.D., Head of the Immunochemistry Laboratory, Institut de Recherches sur le Cancer, B. P. 8, Villejuif (Seine), France

Dan H. Campbell, M.D., Professor of Immunochemistry, California Institute of Technology, Pasadena, California

Charles Christian, The National Foundation, 800 Second Avenue, New York, New York

Charles G. Cochrane, M.D., Division of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California

Frank J. Dixon, M.D., Head, Division of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California

Sheldon Dray, M.D., Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda 14, Maryland

Gerald M. Edelman, M.D., Ph.D., Assistant Professor and Assistant Dean, The Rockefeller Institute, New York 21, New York

John L. Fahey, M.D., National Cancer Institute, National Institutes of Health, Bethesda 14, Maryland

Richard Farr, M. D., Division of Allergy, Scripps Clinic and Research Foundation, La Jolla, California

Joseph D. Feldman, M.D., Division of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California

Marvin Fishman, Ph.D., Division of Applied Immunology, The Public Health Research Institute of the City of New York, Inc., Foot of East 16th Street, New York 9, New York

Edward C. Franklin, M.D., Department of Medicine, New York University School of Medicine, 550 First Avenue, New York 16, New York

Hugh Fudenberg, M.D., Department of Medicine, University of California School of Medicine, San Francisco Medical Center, San Francisco, California

Burton Goldberg, M.D., Department of Pathology, New York University School of Medicine, 550 First Avenue, New York 16, New York

Robert A. Good, M.D., Professor of Pediatrics, University of Minnesota, The Medical School, Heart Hospital Research Laboratory, Minneapolis 14, Minnesota

Howard C. Goodman, M.D., Head, Clinical Immunology Section, Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda 14, Maryland

J. L. Gowans, M.D., Sir William Dunn School of Pathology, University of Oxford, Oxford, England

Pierre Grabar, Professor, Institut Pasteur, 25 Rue du Docteur Roux, Paris, France

Morten Harboe, M.D., University of Oslo, Ullevall Hospital, Institute for Experimental Medical Research, Oslo, Norway

William J. Harrington, M.D., Washington University School of Medicine, St. Louis, Missouri

Milan Hašek, M.D., Czechoslovak Academy of Sciences, Institute of Experimental Biology and Genetics, Prague 6, Czechoslovakia

Walter Heymann, M.D., Associate Professor of Pediatrics, Western Reserve University School of Medicine, Cleveland, Ohio

Halsted R. Holman, M.D., Professor of Medicine, Department of Medicine, Stanford University School of Medicine, Palo Alto, California

John H. Humphrey, M.D., Medical Research Council, National Institute for Medical Research, The Ridgeway, Mill Hill, London N.W. 7, England

James H. Jandl, M.D., Thorndike Memorial Laboratory, Boston City Hospital, Boston 18, Massachusetts

Elvin Kabat, Ph.D., Neurological Institute, 710 W. 168th Street, New York 32, New York

Melvin H. Kaplan, M.D., Cleveland Metropolitan General Hospital, 3395 Scranton Road, Cleveland 9, Ohio

Richard M. Krause, M.D., Washington University School of Medicine, St. Louis, Missouri

Henry G. Kunkel, M.D., Hospital-Rockefeller Institute, New York 21, New York

John P. Leddy, M.D., The University of Rochester, School of Medicine, Rochester 20, New York

F. Gilbert McMahon, M. D., The Upjohn Company, Kalamazoo, Michigan

T. Makinodan, M.D., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Carlos Martinez, M.D., University of Minnesota Medical School, Minneapolis 14, Minnesota

Peter A. Miescher, M.D., Professor of Medicine, New York University School of Medicine, 550 First Avenue, New York 16, New York

Felix Milgrom, M.D., Department of Bacteriology and Immunology, The University of Buffalo School of Medicine, Buffalo 14, New York

Henry Z. Movat, M.D., Associate Professor, Department of Pathology, University of Toronto, Toronto 2, Canada

Hans J. Müller-Eberhard, M.D., The Rockefeller Institute, New York 21, New York

Gus J. V. Nossal, M.D., Deputy Director (Immunology), The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia

Abraham G. Osler, Ph.D., Associate Professor of Microbiology and Medicine, The John Hopkins University School of Medicine, Baltimore 5, Maryland

Zoltan Ovary, M.D., Associate Professor, Department of Pathology, New York University School of Medicine, 550 Frist Avenue, New York 16, New York

E. F. Pfeiffer, Associate Professor of Medicine, 1. Medizinische Universitätsklinik, Frankfurt/Main, 14 L. Rehn-Strasse, Germany

David Pressman, Ph.D., Director of Cancer Research in Biochemistry, Roswell Park Memorial Institute, Buffalo 3, New York

Jonas Salk, M.D., Salk Institute for Biological Research, La Jolla, California

Beatrice C. Seegal, M.D., College of Physicians and Surgeons, Columbia University, New York 32, New York

Michael Sela, M.D., Associate Professor, Department of Biophysics, The Weizmann Institute of Science, Rehovath, Israel

N. Raphael Shulman, M.D., Chief, Clinical Hematology Branch, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda 14, Maryland

Sidney Shulman, M.D., University of Buffalo, Buffalo, New York

Hans Spiegelberg, M.D., Department of Medicine, New York University School of Medicine, 550 First Avenue, New York 16, New York

Raymond W. Steblay, M.D., The University of Chicago, Department of Obstetrics and Gynecology, Chicago 37, Illinois

Jaroslav Sterzl, M.D., Head, Department of Immunology, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague 6, Czechoslovakia

David W. Talmage, M.D., University of Colorado Medical Center, Denver 20, Colorado

Jonathan W. Uhr, M.D., Associate Professor of Medicine, New York University School of Medicine, 550 First Avenue, New York 16, New York

J. J. van Loghem, Professor of Immunopathology, Director, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Plesmanlaan 125, Amsterdam, Netherlands

John H. Vaughan, M.D., Associate Professor of Medicine, The University of Rochester School of Medicine, Rochester 20, New York

Jacinto J. Vazquez, M.D., Division of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California

William O. Weigle, Ph.D., Division of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California

Ernest Witebsky, M.D., Chairman, Department of Bacteriology and Immunology, University of Buffalo Medical School, Buffalo, New York

Morris Ziff, M.D., Department of Internal Medicine, The University of Texas, Southwestern Medical School, Dallas 35, Texas

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I. Structure and Genetics of Immune-Globulins

II. Antibody Formation

III. Influence of Various Factors on Antibody Response

IV. Mechanisms of Immunogenic Kidney Diseases

V. Mechanisms of Blood Cell Damage Produced by Immunological Reactions

Genetically Determined Structures of Immune-Globulins[1]

Morten Harboe[2] *and C. Kirk Osterland*[3]

The Rockefeller Institute, New York, N.Y.

Antigenic differences between the γ-globulins of different individuals within a given species have been demonstrated in the rabbit [6, 7, 36, 37], mouse [26], guinea pig [2] and man [16, 17, 25]. γ-Globulin factors in man and the rabbit will be considered here since these are the two species most carefully studied and a comparison between the two appears useful.

At least seven different γ-globulin factors have been defined in man and their genetic basis clearly established [3, 17, 18, 20, 41, 43, 44]. These factors are determined by genes present at two separate loci, the *Gm* and *Inv* loci [41, 42]. In whites, factors Gm(a) and Gm(b) are determined by two genes which behave as alternate alleles [18] and the same is true for the Inv(a) and Inv(b) factors [44]. All the factors are determined by hemagglutination inhibition reactions where the test system consists of Rh positive red cells coated with selected incomplete anti-Rh antibodies and selected sera which are able to agglutinate such cells. Inhibition of this type of agglutination means presence of the genetic factor.

In rabbits, similar genetically determined factors are generally known as allotypes [36, 37, 6a]. These appear to be controlled by six genes present at two distinct loci. In a recently suggested notation for allotypy [6a], the six factors are referred to as A1, A2, A3, A4, A5 and A6. The genes which control A1, A2 and A3 are present at locus *a*, and those controlling A4, A5 and A6 at locus *b*.

7S and 19S γ-globulins, β_{2A}-globulin and Bence Jones proteins are structurally and functionally closely related. The cause of the immunological cross-reactions between these proteins has been clarified through the use of enzymatic digestion and characterization of the resulting fragments. By splitting of human γ-globulin with papain in the presence of cysteine, two main antigenically distinct fragments are obtained which have been called "slow" (S) and "fast" (F) because of different electrophoretic mobility [9]. By chromatographic separation, three fragments are

[1] Aided by a grant from The National Foundation.

[2] Present address: Institute for Experimental Medical Research, Ullevaal Hospital, Oslo, Norway.

[3] Fellow, Canadian Arthritis Foundation.

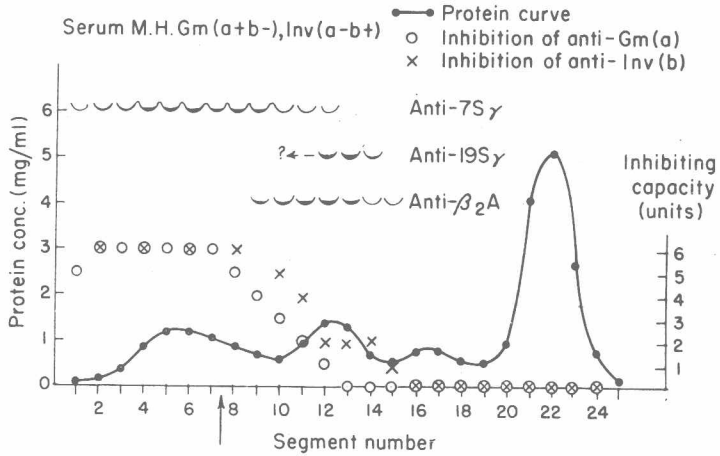

Fig. 1. Zone electrophoresis of a normal serum to show distribution of the Gm(a) and Inv(b) characters. The proteins of each segment were brought to the same concentration before testing. The antigenic character of the proteins was determined by agar diffusion tests using specific antisera as indicated by the lines in the upper portion of the curve. The term "inhibiting capacity (units)" refers to the number of doubling dilutions which completely inhibited the agglutination of the respective test systems starting at a concentration of 1 mg/ml.

obtained of which A and C in *Franklin*'s nomenclature [13] are immunologically identical and correspond to S, whereas the third fragment, B, corresponds to F. The cross-reaction between 7S γ-globulin and the other proteins is due to antigenic determinants present on the S fragment which also are present on the other proteins [1, 12, 14, 24]. In contrast, the major antigenic determinants characteristic of the F part of 7S γ-globulin are only present on this protein.

7S and 19S γ-globulins, β_{2A}-globulins and Bence Jones proteins isolated from patients with tumors of the plasma cell series were studied for the presence of Gm and Inv characters [22]. Gm factors were found only on 7S γ-globulin, whereas Inv factors were found on all four types of proteins. A correlation was observed between the type of Bence Jones proteins [5, 27, 30] and the finding of genetic characters since only Bence Jones proteins of type I [30] were Inv(a) or Inv(b) positive. In contrast, 7S γ-myeloma proteins of both type I and II [30] were found to possess Inv characters.

A similar distribution of genetic characters was observed in the corresponding normal proteins. Fig. 1 shows the findings after zone electrophoresis of a normal serum and testing of various fractions for the Gm(a) and Inv(b) factors. It may be seen that the distribution of Gm(a) activity corresponded to the distribution of 7S γ-globulin as determined by precipitin studies with absorbed antisera reacting specifically with 7S γ-globulin. Inv(b) activity was found in this area and further towards the cathode corresponding to the distribution of 19S γ-globulin and β_{2A}-globulin.

Table I

Genetic types of 7S and 19S γ-globulins purified from six normal sera

Serum	7S γ-globulin		19S γ-globulin	
1.	Gm(a+b+)	Inv(a+b+)	Gm(a—b—)	Inv(a+b+)
2.	Gm(a+b+)	Inv(a+b+)	Gm(a—b—)	Inv(a+b+)
3.	Gm(a+b+)	Inv(a—b+)	Gm(a—b—)	Inv(a—b+)
4.	Gm(a+b+)	Inv(a—b+)	Gm(a—b—)	Inv(a—b+)
5.	Gm(a+b+)	Inv(a—b+)	Gm(a—b—)	Inv(a—b+)
6.	Gm(a—b+)	Inv(a—b+)	Gm(a—b—)	Inv(a—b+)

The 7S and 19S γ-globulins were purified from six individual normal sera by density gradient ultracentrifugation of euglobulin preparations [21] and tested for Gm and Inv factors. Table I shows that Gm factors were found on 7S γ-globulin, whereas all the 19S γ-globulin preparations were Gm(a—b—). In contrast, the Inv type of the 7S and 19S γ-globulins from the same serum was identical in all instances indicating that a part of the 7S and 19S γ-globulin molecules are under the same genetic control in normal individuals.

The finding of Gm factors only on 7S γ-globulin and Inv factors on all four types of protein together with the known structural relationships between these proteins suggested that the Gm determining sites are located on the F part of 7S γ-globulin and that the Inv sites are present on the S part of the molecule. This was shown to be the case both in 7S γ-globulins isolated from individual normal sera, in Fraction II γ-globulin prepared from pooled human plasmas and in myelo-

Table II

Gm(a) typing of a papain split 7S γ-myeloma protein

Dilution of Material	Whole Split	S	F	Normal γ-globulin (control)	
				Gm(a+)	Gm(a—)
neat (1 mg/ml)	0	3	0	0	3
1:2	0	3	0	0	3
1:4	0	3	0	0	3
1:8	0	3	0	0	3
1:16	0	3	0	0	3
1:32	0	3	0	0	3
1:64	1	3	1	1	3

Degree of agglutination recorded 3, 2, 1. 0 = no agglutination.

Reagents: Anti-Gm(a) Smejsa diluted 1:35
Group 0 Rh pos. red cells coated with anti-D J. J.

Controls: Anti-Gm(a) + saline + coated cells: 3
Anti-Gm(a) + saline + uncoated cells: 0
Coated cells + saline: 0
The protein preparations + saline + coated cells: 0

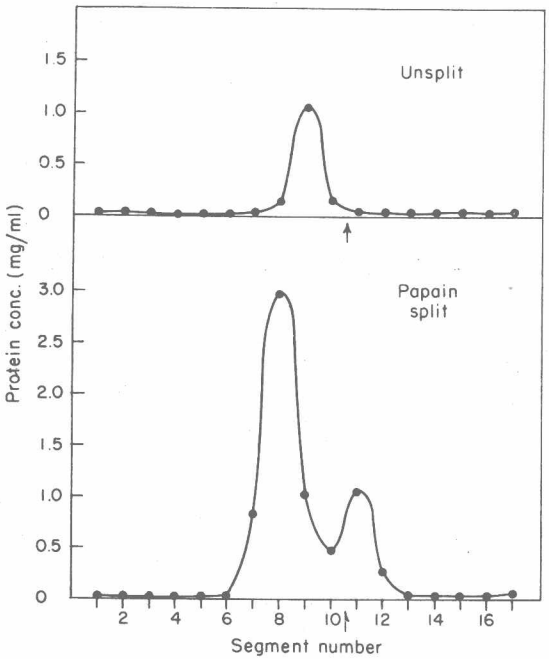

Fig. 2. Zone electrophoresis of an isolated γ-myeloma protein and its papain digest to show isolation of the S and F fragments.

ma proteins of the 7S γ-type. The findings on the latter will be used as an example to illustrate our techniques. γ-Myeloma proteins of low electrophoretic mobility were isolated by zone electrophoresis on starch and subjected to splitting by papain in the presence of cysteine at pH 7.4 [40]. Zone electrophoresis of the digest gave two sharp peaks as shown in Fig. 2. In this case the protein of segment 7 constituted the S preparation, and that of segment 12 the F preparation. Both were immunologically pure [21]. The preparations were tested for the presence of genetic factors. Table II shows the results of tests for the Gm(a) factor. The reactions of control preparations of Gm(a+) and Gm(a—) normal γ-globulins are included for comparison. Gm(a+) γ-globulin inhibited the agglutination completely in six doubling dilutions starting at a concentration of 1 mg/ml whereas Gm(a—) γ-globulin lacked inhibiting capacity at a concentration of 1 mg/ml. The isolated myeloma protein and its papain digest were both strongly inhibiting. After isolation of the fragments the F fragment inhibited the agglutination, whereas the S fragment had no inhibiting capacity at a concentration of 1 mg/ml. Similar findings were made with regard to the Gm(x) and Gm(b) characters: inhibiting activity was confined in all instances to fractions containing the F fragment, whereas immunologically pure S preparations had no inhibiting capacity. It was noted in