BASIC IMMUNOGENETICS

Third Edition

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New York Oxford
OXFORD UNIVERSITY PRESS
1984

Library of Congress Cataloging in Publication Data
Main entry under title:

Basic immunogenetics.

Bibliography: p.
Includes index.
1. Immunogenetics. I. Fudenberg, H. Hugh.
[DNLM: 1. Immunogenetics. QW 541 B311]
QR184.B37 1984 616.07'9 83-23738
ISBN 0-19-503404-X
ISBN 0-19-503405-8 (pbk.)

Printed in the United States of America

Printing (last digit): 9 8 7 6 5 4 3 2 1

Preface to First Edition

Inherited differences among individuals, or genetic polymorphisms, have frequently been studied by immunological methods. This field of study can loosely be called immunogenetics. Rather than choosing to deal with the whole range of immunogenetics in this text, we have concentrated our attention on areas where the polymorphisms seem to be of particular importance—either because they have been studied in detail (as in the case of some human blood group antigens), because they reveal a wide spectrum of genetic mechanisms operative in producing genetically determined antigens, or because they may raise general questions about individual variations or about regulation of the immune response per se (that is, immune response genes). In this last category are the immunoglobulin polymorphisms, to which we have devoted a large part of the text. Unavoidably, the choice of subjects reflects the writers' own research interests; however, we hope that the examples we have chosen are sufficient to illustrate the variety and potential complexity of other polymorphic systems.

At the end of each chapter is a short bibliography, often containing references to review articles rather than an extensive list of original references. In each chapter subjects are introduced at a level intended not for the specialist but for graduate and medical students with a fair knowledge of biochemistry and genetics. For this reason, many important results have been presented without mention of the authors' names: we apologize in advance to the numerous authors who are not directly acknowledged in the text.

Portions of an article by Dr. J. R. L. Pink that appeared in the *New Scientist* were adapted for use in the Introduction.

H.H.F.
J.R.L.P.
D.P.S.
A.-C.W.

Preface to Third Edition

The third edition of this book has tried to incorporate most of the enormous advances in the field of immunogenetics since the publication of the second edition. Each chapter has been extensively revised in an attempt to cogently present such emerging concepts as immunoglobulin gene rearrangement, evolution, and antibody diversity. Chapter 5 has undergone a major overhaul, reflecting the great increase in knowledge of membrane antigens that occurred subsequent to monoclonal antibody analysis.

A new chapter has been added (Chapter 7) on Immunodeficiency that emphasizes the diversity of the immunological repertoire and the dangers of its dysfunction on homeostasis. Both the glossary of immunologic terms and the blood group terminology have been updated.

It is hoped that this book will serve not only as a reference book for those interested in immunogenetics, but also as an appropriate tool for instructing students, both undergraduate and graduate, in health-related fields.

We are most grateful to Mrs. Linda Paddock and Nancy Butler for superb secretarial assistance. In addition, a special debt of gratitude is owed to Drs. F. Esposito and H. D. Whitten for helping to prepare special crucial sections of the book.

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G.B.F.

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CHAPTER 1

Introduction

Vertebrates, from primitive fish to humans, possess a well-organized system for defending themselves against infection. When an antigen—a collective term for intruding "foreign" macromolecules such as bacterial coat proteins—enters the bloodstream or lymph, it induces the formation of protective antibody proteins by some of the body's immunocytes. Antibodies are capable of combining with, and usually of precipitating or inactivating, the offending antigen. Figure 1.1 shows antibody molecules (from the high-molecular-weight protein fraction of cow blood) combining with a bacterial flagellum; the antibodies appear rather like staples stuck along the length of the antigen. In the normal course of events, the aggregate formed by the antigen–antibody reaction is removed by the body's scavenging (phagocytic) cells or destroyed by antibodies with the aid of the hemolytic complement system (a mixture of at least nine components, present in the blood, whose destructive action toward bacterial or other cells is triggered by the formation of the antigen–antibody complex).

Antibodies are remarkable for their ability to distinguish antigenic macromolecules from normal body constituents. In addition they can distinguish very subtle differences among the intruding antigens. The high specificity of different antibody preparations was first clearly demonstrated by the German chemist Karl Landsteiner; he found, for example, that rabbit antibodies can distinguish between two antigenic proteins differing only in that a D-tartaric acid residue is attached to one, and its optical isomer, an L-tartaric acid residue, to the other. Figure 1.2 shows some of the compounds used by Landsteiner in his classical examination of the specificity of different antibody preparations.

Fig. 1.1 Antibodies from cow serum in combination with a bacterial flagellum. The antibodies appear like staples in this photograph; they are of the IgM class. Magnification × 356,000. (Courtesy of Dr. A. Feinstein)

The differences between these compounds are so slight that the number of different antibodies an individual can make must be very large—perhaps of the order of a million or more.

These considerations raised the first of many puzzles that have intrigued immunologists. The antibodies produced in response to various antigens appeared to differ significantly from each other not in gross physical or chemical characteristics, but only in ability to combine with the different antigens. What makes one antibody different from another? Are the many different antibodies genuinely different proteins (that is, proteins with different amino acid sequences), or are they proteins with identical sequences but with the ability to fold around antigens in a great variety of ways?

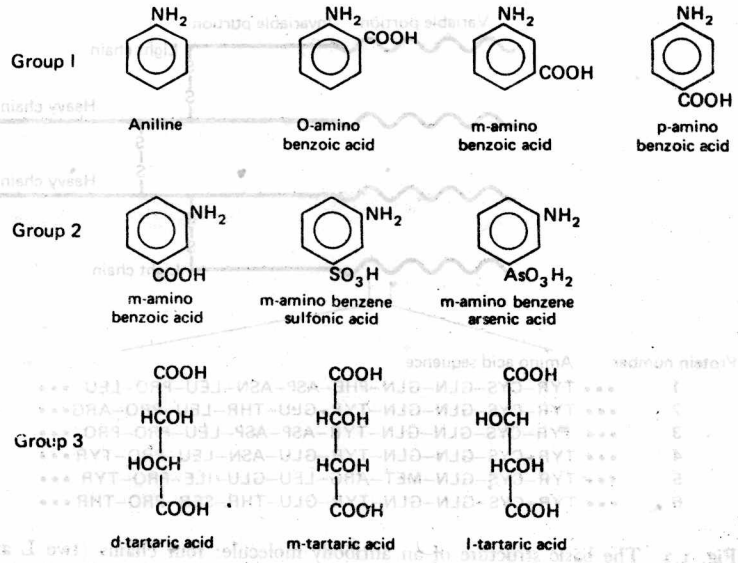
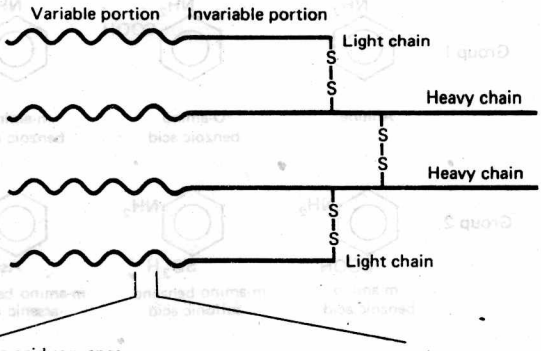


Fig. 1.2 Some groups of compounds used by Landsteiner to demonstrate the high specificity of antibody preparations. By themselves, low-molecular-weight compounds are generally incapable of inducing antibody synthesis, but if the compounds are chemically coupled to protein molecules (in which form they are described as haptens) and injected into an experimental animal, antibodies against the hapten (as well as the protein) will be made. Landsteiner observed that antibodies against each hapten (of the groups shown here) were quite distinct, giving no reaction with the other chemically related hapten.

This problem has been resolved: different antibody molecules do have different sequences. However, the question was difficult to answer, for the following reason: almost all antibody preparations examined to date have been heterogeneous. As discussed in Chapter 2, the evolution of the antibody protein system has produced a family of protein chains—of at least 10 distinct types in humans—many or all of which are present in typical antibody preparations. Even within one individual, each type includes an enormous number of variants differing slightly from each other in sequence. These chains can combine with each other in different ways to give variations of the basic antibody protein structure shown in Figure 1.3. The proteins formed in this manner are defined as the immunoglobulins.

Chapter 3 elaborates on a further cause of heterogeneity in anti-



Protein number	Amino acid sequence
1	••• TYR–CYS–GLN–GLN–PHE–ASP–ASN–LEU–PRO–LEU •••
2	••• TYR–CYS–GLN–GLN–TYR–GLU–THR–LEU–PRO–ARG•••
3	••• TYR–CYS–GLN–GLN–TYR–ASP–ASP–LEU–PRO–PRO •••
4	••• TYR–CYS–GLN–GLN–TYR–GLU–ASN–LEU–PRO–TYR •••
5	••• TYR–CYS–GLN–MET–ARG–LEU–GLU–ILE–PRO–TYR •••
6	••• TYR–CYS–GLN–GLN–TYR–GLU–THR–SER–PRO–THR •••

Fig. 1.3 The basic structure of an antibody molecule: four chains (two L and two H) joined by disulfide bridges. The amino-terminal portion of each chain (the V region) differs in different antibodies, while the remainder of the chain (the C region) may be identical in different antibodies.

body preparations: genetic differences (inherited in a Mendelian fashion) may distinguish the immunoglobulins of one individual from those of another. These differences (known as "allotypic" alternatives) have been very helpful in analyzing the genetic basis of antibody variability, since the presence of a genetic marker directly associated with differences in protein structure (in an otherwise homogeneous protein chain) provides good evidence that a single gene (stretch of DNA) codes for that sequence.

These results, although answering previous questions about the structural basis of antibody variability, raise another problem: how is the genetic information necessary to code for perhaps a million different antibodies stored in the body and transmitted from one generation to another? Is there a separate gene for each one of the possible antibody sequences? Is the information stored as single master stretches of DNA that can be altered by mutation during the life of an individual (or perhaps decoded in a number of different ways)? Is the information coded in a few stretches of DNA that recombine among themselves to give many different combinations of

sequence? The surprising result of the genetic and structural studies described in Chapters 3 and 4 was that each immunoglobulin chain consists of a variable (V) region, in which the amino acid sequence variability responsible for antibody specificity is located, and a constant (C) region (Fig. 1.3 and Table 1.1). The DNA sequences coding for V and C regions are separate, and a fascinating variety of different mechanisms, discussed in Chapter 4, contributes to generating V-region diversity.

These studies on the genetic control of antibody synthesis naturally raise a further question, discussed in Chapter 5: what are the genetic bases of the many diseases that involve a defect in antibody synthesis? These defects fall into two classes. First, there may be a specific defect in the ability to respond to a particular antigen—for example, some strains of mice or guinea pigs cannot produce an immune response to certain synthetic antigens—a phenomenon subsequently observed in humans with natural allergens. Alternatively, there may be a general lack of immune function—that is, an inability to synthesize any antibodies, or to reject tissue transplants, or both. Such defects, either "antigen-specific" or generalized, are found in certain rare human diseases, most of which clearly have a genetic origin.

One might expect that genes responsible for antigen-specific defects in immune responses would code for immunoglobulin V regions, but in fact only rarely has this correlation been observed. Much more frequently, the genes responsible for antigen-specific immune response defects have been located in a single chromosomal region called the major histocompatibility complex (MHC). This region controls the synthesis of a variety of immunologically relevant proteins, including (1) certain complement components, (2) the major histocompatibility antigens, which are important in defense against viral infections, and (3) the antigens coded by the *Ir* (immune response) genes, to which many antibody response defects have been localized.

In order to understand how the products of these genes function, we need to know in detail how the synthesis of a particular antibody is induced by the presence of the corresponding antigen. Much evidence shows that each antibody-producing cell, with few if any exceptions, makes antibody of only one specificity and, therefore, has been committed to using only one V-region gene for L-chain synthesis and one for H-chain synthesis. Thus individual antibody-synthesizing cells taken from a mouse that has been immunized

Table 1.1 Comparison of the complete amino acid sequences of two human Bence–Jones proteins (from patients Ha and Bo). The sequence of protein Ha is given in full, and only the positions where protein Bo differs in sequence are indicated. The chains are of the λ type, but similar variation between proteins is observed in κ and H chains.' Comparison of several λ chains indicates that sequence variation in these chains is observed between positions 1 and 107 (the V region). The remainder of the chain constitutes the C region. PCA is pyrrolidone carboxylic acid (a cyclized glutamate derivative).

1								10									20						
PCA	Ser	Val	Leu	Thr	Gln	Pro	Pro	Ser	Val	Ser	Gly	Thr	Pro	Gly	Gln	Arg	Val	Thr	Ile	Ser	Cys	Ser	Gly	Gly
		Ala							Ala			Ser				Ser						Thr		Thr
				30											40									50
Ser	Ser	Asn	Gly	Thr	Gly	Asn	Asn	Tyr	Val	Tyr	Trp	Tyr	Gln	Gln	Leu	Pro	Gly	Thr	Ala	Pro	Lys	Leu	Leu	Ile
		Asp	Val	Gly	Asn	Asp	Lys			Ser					His			Arg					Val	
								60											70					
Tyr	Arg	Asp	Asp	Lys	Arg	Pro	Ser	Gly	Val	Pro	Asp	Arg	Phe	Ser	Gly	Ser	Lys	Ser	Gly	Thr	Ser	Ala	Ser	Leu
Phe	Glu	Val	Ser	Gln															Asn	Asp	Thr			
				80											90									100
Ala	Ile	Ser	Gly	Leu	Arg	Ser	Glu	Asp	Glu	Ala	His	Tyr	His	Cys	Ala	Ala	Trp	Asp	Tyr	Arg	Leu	Ser	Ala	Val
Thr	Val					Ala					Asp		Tyr		Ser	Ser	Tyr	Val	Asp	Asn	Asn	Asn	Phe	?
								110											120					
Val	Phe	Gly	Gly	Gly	Thr	Gln	Leu	Thr	Val	Leu	Arg	Gln	Pro	Lys	Ala	Ala	Pro	Ser	Val	Thr	Leu	Phe	Pro	Pro
						Lys																		
				130											140									150
Ser	Ser	Glu	Glu	Leu	Gln	Ala	Asn	Lys	Ala	Thr	Leu	Val	Cys	Leu	Ile	Ser	Asp	Phe	Tyr	Pro	Gly	Ala	Val	Thr
									160										170					
Val	Ala	Trp	Lys	Ala	Asp	Ser	Ser	Pro	Val	Lys	Ala	Gly	Val	Glu	Thr	Thr	Thr	Pro	Ser	Lys	Gln	Ser	Asn	Asn
				180											190									200
Lys	Tyr	Ala	Ala	Ser	Ser	Tyr	Leu	Ser	Leu	Thr	Pro	Glu	Gln	Trp	Lys	Ser	His	Arg	Ser	Tyr	Ser	Cys	Gln	Val
									210															
Thr	His	Glu	Gly	Ser	Thr	Val	Glu	Lys	Thr	Val	Ala	Pro	Thr	Glu	Cys	Ser								

with two antigens (for example the easily distinguishable red blood cells from sheep and from camels) do not make antibodies to both antigens. In addition the types of antibody-producing cells found in myelomatosis,* which are presumed to be derived from a single parent cell, also produce antibody-like molecules of unique sequence.

These findings are incorporated in a theory of antibody production first advocated by Jerne and by Macfarlane Burnet (the clonal selection theory). According to this theory in its simplest form, antigen can induce antibody synthesis only by contact with a small proportion of cells (antigen-sensitive cells) that have already been committed to making that antibody (Fig. 1.4). The process of commitment of these cells, which takes place during their development and differentiation in the immune system, is still mysterious, but it presumably involves the synthesis of an antibody-like "receptor" molecule that is carried on the cell's surface and that, on contact with antigen, can stimulate the cell to divide and differentiate. The "receptor" has a specificity identical to that of the antibody that the cell and its progeny are destined to secrete. Combination of a receptor with antigen is presumably a trigger that stimulates the antigen-sensitive cell to divide and produce a large number (a "clone") of daughter cells. Cells of a single clone produce antibody molecules of identical structures, but the type of antibody produced by each clone may be different.

These ideas may be used to explain many different observations. However, a variety of experimental results cannot be explained by the clonal selection theory in this simple form. For example, initiation of an immune response, rather than resulting from combination of antigen with single antigen-sensitive cells, in some cases requires the cooperation of more than one cell in recognizing the antigen. It has been shown, on the one hand, that, to provoke an antibody response, such an antigen must have at least two recognizable determi-

* In certain disorders of antibody-producing cells, homogeneous immunoglobulins, for the most part without obvious antibody activity, are produced in the blood of individuals suffering from a malignant proliferation of cells in the bone marrow. In humans, the classical examples of such diseases are (1) Waldenström's macroglobulinemia, characterized by overproduction of homogeneous IgM in the serum and by proliferation of lymphoid cells, and (2) myelomatosis, characterized by overproduction of any of the other immunoglobulin classes (IgG, IgA, etc.) and proliferation of plasma cells with appearance of destructive lesions in bone tissue. In some strains of mice, tumors that secrete immunoglobulins can be induced experimentally (by injecting the mice with certain mineral oils.)

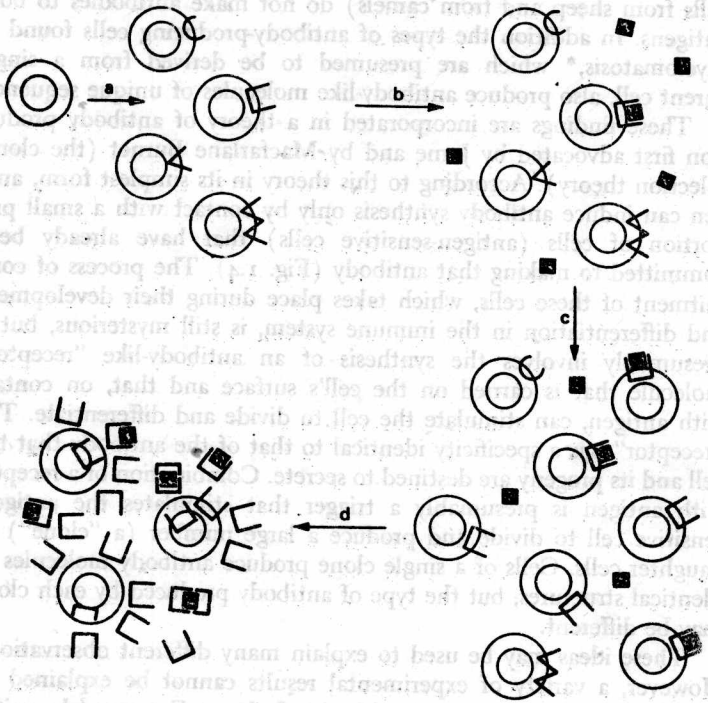


Fig. 1.4 A simplified version of the theory of clonal selection. (a) During development of an individual, lymphoid cells mature to give antigen-sensitive cells carrying antibody-like receptors (V VV U C) of many different specificities. (b) A foreign antigen (■) comes into contact with a cell carrying a receptor complementary to that antigen. (c) This cell proliferates to give a clone of identical cells, and (d) cells of this clone differentiate and secrete antibody of a single specificity. If the antigen is encountered a second time on a later occasion, more antigen-sensitive cells are available to proliferate, and these will produce an increased (secondary or anamnestic) immune response to that antigen.

nants on its surface, and, on the other hand, that antigen recognition leading to antibody production (at least for certain antigens) probably requires the interaction of at least two separate lymphoid cell populations. One population (probably derived directly from the bone marrow) is responsible for antibody production, and the other undergoes differentiation in the thymus and is at least partly responsible for antigen recognition and for some specific immunological functions (such as graft rejection) whose exact mechanisms are still unknown. This duality is paralleled by the empirical division of immunological reactions into those mediated by antibody molecules present in the serum or in secretions (humoral immunity) and those —including graft rejection—that are mediated by cells but that, because of their specificity, are thought to depend on the presence of antibody-like molecules on the surfaces of these cells (cellular immunity). Chapter 5 deals with the possibilities of using these results to account for genetic aberrations in antibody synthesis, and with the role of the major histocompatibility complex in cell–cell interactions.

Many of the findings we have mentioned depended on the use of concepts or techniques borrowed from fields not directly related to immunology. For example, the techniques used to determine the amino acid sequence of an immunoglobulin molecule and the concepts that helped relate this structure to the function and evolution of the molecule (Chapter 2) were, for the most part, developed in the course of work on insulin, hemoglobin, ribonuclease, and other proteins investigated by molecular biologists. The mapping of the immunoglobulin gene linkage groups (Chapter 3) has involved using the techniques of population genetics, serology, protein chemistry and molecular biology. These studies could not have been carried out without the results from clinical and chemical laboratories leading to characterization of myelomatosis and isolation of the proteins produced in the course of the disease.

Several other techniques have recently resulted in crucial advances in immunogenetics, including assortive breeding leading to the development of congenic, coisogenic, and recombinant inbred strains of experimental animals (Chapter 5); nucleic acid hybridization, whereby enzymatically cleaved strands of nucleic acid are allowed to react with a radiolabeled DNA or RNA probe fragment (Chapter 4); nucleic acid sequencing, whereby individual nucleic acid bases may be sequenced after modification and cleavage on polyacrylamide gel electrophoresis; recombinant DNA cloning, the process whereby

an isolated gene is inserted into a vector plasmid or bacteriophage prior to transformation or infection of a bacterial host; and gene mapping by endonuclease cleavage—a very precise process in which cleavage of DNA segments by a series of different, specific endonucleases allows the determination of the order of the gene map.

Immunology is already repaying this debt owed to many other disciplines. The concepts (1) that two or more genes may code for a single polypeptide chain, (2) that families of multiple, closely linked, closely related genes may be basic units of vertebrate genomes, and (3) that gene rearrangement may occur during differentiation are now central to eukaryotic molecular biology. The techniques of radioimmunoassay, tissue typing, and monoclonal antibody production are widely used in many fields of both basic and clinical research. The further development of immunology will, we hope, continue to prove useful in many biological fields and in the provision of new methods for eradication, prevention, diagnosis, or treatment of human disease.

RECOMMENDED READINGS

Books

Klein, J. (1982), *Immunology, The Science of Self–Nonself Discrimination* (1st ed.). New York: Wiley, pp. 156–255.

Wachtel, S. S. (1982), *H-Y Antigen and the Biology of Sex Determination*. New York: Grune & Stratton.

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