CLINICAL IMMUNOLOGY

Editors

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PREFACE

As a result of the continuing rapid accumulation of new knowledge in clinical immunology, this second edition has been almost entirely rewritten with the assistance of ten new contributors and a coeditor. We have added new chapters on immune resistance to infection by microorganisms, immunotherapy and immunosuppression, and immunoassays in clinical medicine. The chapters which appeared in the first edition have been greatly expanded to include recent advances in fundamental and applied immunology, as well as a more detailed treatment of basic immunopathologic mechanisms. The discussion of bronchial asthma, especially, has been enlarged and divided into two chapters to provide adequate coverage of the burgeoning new data pertinent to IgE-mediated hypersensitivity in the lung.

Almost every clinical discipline has been influenced by the information explosion that has taken place in the science of immunology over the past fifteen years. CLINICAL IMMUNOLOGY brings recent progress in immunologic research to practicing physicians in a comprehensible and useful fashion. Each contributor has a background in both the experimental and clinical aspects of the topics discussed.

This volume is written not only for specialists in clinical immunology and allergy, but also for clinicians in internal medicine, pediatrics, laboratory medicine, and surgery. It will be of particular assistance to physicians preparing for board examinations in allergy and immunology and to students wishing to acquire a specialized knowledge of applied immunology.

The selection of topics for inclusion in a textbook of this type must of necessity reflect the opinions of the editors since the scope of clinical immunology is relatively new and loosely defined. An attempt has been made to stress those disease processes which are encountered most frequently in clinical practice or which illustrate an important immunologic concept. Chapters 1 and 2 introduce those immunologic principles having the greatest relevance to the clinical chapters that follow; they are not intended to be an exhaustive review of basic immunology. The results of animal experimentation are discussed only when they are considered essential to the understanding of comparable human disorders.

The clinical chapters which follow present a broad viewpoint of immunologic disorders, including the atopic and connective tissue diseases, diseases associated with hypergammaglobulinemia and autoantibodies, immunologic deficiency states, immunhematology, organ transplantation, cancer immunology, hypersensitivity to drugs, and the immunologic aspects of systemic disease. Laboratory tests relevant to clinical immunology are presented in the final two chapters.

CLINICAL IMMUNOLOGY is written in a manner that is intelligible to those reading in the area for the first time, yet it does not sacrifice accuracy through oversimplification. Nine of the fourteen contributors are members of the Division of Clinical Immunology of the Montreal General Hospital and the other five collaborate closely with the Division in its teaching, research, and clinical activities, which we hope will add a cohesiveness and absence of duplication frequently

missing in multiauthored texts. The editor of the first edition (S.O.F.) is proud to welcome Phil Gold as coeditor. His unique professional background and encyclopedic grasp of modern immunology has enriched the text throughout.

For assistance in the preparation of this volume we are grateful to a large number of colleagues, residents, research fellows and students for many helpful suggestions. In particular, we wish to acknowledge the invaluable contributions made by Drs. J. M. Esdaile, A. Fuks, K. E. MacLaughlin, J. I. Schulz and H. C. G. Wong in the preparation of Chapter 16; and the assistance of Dr. A. Gutkowski in compiling the data for Tables 20–4, and 20–5. For critically reviewing specialized portions of the manuscript we wish to express our gratitude to the following colleagues at McGill University: Drs. R. D. Guttmann, D. G. Haegert, W. S. Lapp, R. Mangel and E. A. Shaffer.

One of our greatest debts is to our office staff, Mrs. Lorraine Habib, Mrs. Mary Kerrigan and Miss Karen Matheson who toiled long hours typing numerous revisions of the manuscript. We also wish to thank Mr. H. Artinian and Mrs. Beverley Lehman of the Department of Audio-Visual Services of the Montreal General Hospital for the preparation of illustrations and figures; and Mrs. Joan Kwiecinska and Mrs. Carol Laxer of the Medical Library of the Montreal General Hospital for countless hours spent in the procurement and verification of references. Mr. Daniel Freedman assisted with much of the proofreading.

The unfailing cooperation of the staff of the Medical Department of Harper & Row has made the task of preparing the second edition a most pleasant and stimulating experience.

To our wives, Norah and Evelyn, and our children, we can only express our deepest appreciation for their interest and patience while the manuscript was in preparation. Without their constant support and encouragement, this book would not have been possible.

S.O.F.
P.G.

Montreal, Canada

CONTENTS

THE BIOLOGY OF THE IMMUNE RESPONSE

1

PATRICIA A. L. KONGSHAVN, DAVID HAWKINS, JOSEPH SHUSTER

WHAT IS CLINICAL IMMUNOLOGY?

Immune reactions can be beneficial to the host (as exemplified by immunization procedures against infectious diseases) or harmful to the host (as exemplified by diseases such as allergic asthma, hay fever or diseases associated with autoantibodies). In either case, the "foreign" substance (antigen) evokes an *immune response* which ultimately results in the synthesis of specifically reactive proteins (antibodies), the production of specifically modified reactive cells, or both. Defined in terms of its most narrow and traditional meaning, *immunology* is the study of *immunity*, and immunity is a state of increased resistance to disease. However, a more modern viewpoint is that immunology is the study of all aspects of the immune response as it affects both man and experimental animals.

The word *allergy*, as originally defined by von Pirquet at the turn of the century, was used to denote a state of altered reactivity which resulted from antigenic exposure and was harmful to the host. For many years afterward, the terms *allergy* and *hypersensitivity* were used almost interchangeably to describe a deleterious clinical response to foreign substances, as opposed to the protective effects of immunity. However, the distinction between immunity and allergy or hypersensitivity is somewhat artificial, as the consequences which result from either of these states are by no means mutually exclusive. For example, the common procedure of smallpox vaccination usually results in a high degree of protective immunity, although a small proportion of individuals may also develop a harmful allergic encephalitis (see Ch. 16). In order to avoid semantic confusion, some authors have attempted to restore the word allergy to its original meaning by the introduction of terms such as autoallergic instead of autoimmune, but this suggestion has not yet been widely accepted.

The *clinical immunologist* is primarily concerned with 1) the elimination of undesired immune reactions in patients who have a wide variety of disease processes with immunologic features, or in patients undergoing organ transplantation; 2) the investigation and management of patients with immune deficiency states, and 3) the production of protective immunity in individuals exposed or potentially exposed to infectious diseases. Thus, the clinical immunologist is a process-oriented medical specialist rather than an organ- or system-oriented specialist as is the case with a cardiologist, ophthalmologist or neurologist.

Ideally, the clinical immunologist should be familiar with both the clinical and laboratory aspects of immunology as they relate to human disease processes. In other words, the role of the clinical immunologist in a modern hospital setting may be considered as that of a physician–scientist who should be proficient in the clinical evaluation of patients with immunologic diseases and trained to perform and interpret common laboratory procedures relevant to clinical immunology. In many institutions, the clinical immunologist often fulfills an important research function in addition to his clinical and laboratory responsibilities. In fact, immunology has always been a branch of medical science in which fundamental advances have led quickly to practical applications of outstanding importance. The effective control of poliomyelitis, the prevention of the tragic consequences of hemolytic disease of the newborn, a new understanding of host resistance to cancer and the continuing progress in organ transplantation are but a few recent examples of the close link between basic research in im-

munology and the eradication of human disease.

ANTIGENS

IMMUNOGENS AND ANTIGENS

Although the terms immunogen and antigen are frequently used interchangeably, they are not necessarily synonymous. *Immunogenicity* may be defined as the capacity of a substance to initiate a humoral or cell-mediated immune response, whereas *antigenicity* may be defined as the capacity of a substance to bind specifically with the antibody molecules whose formation it has elicited. The word *ligand* has also been employed to describe the latter property of specific binding to antibodies. Employed correctly, the term *immunogen* specifies that a substance acts at the afferent limb of the immune response (see section on Immune Response). However, unless otherwise stated, the term *antigen* will be used throughout the text to describe substances which have immunogenic capacity, antigenic capacity, or both.

Most conventional antigens possess both immunogenic and antigenic capacities, although in some instances these may not be apparent due to a state of immunologic unresponsiveness (see section on Immunologic Unresponsiveness), or due to genetic inability of the host to respond to certain antigens. Most immunogens are large molecules such as proteins, polysaccharides, polypeptides and polynucleotides.

SYNTHETIC ANTIGENS

Techniques for the production of synthetic random homo- and copolymers of amino acids were developed a number of years ago and these compounds have proved particularly useful for studying the molecular basis of antigenicity and the genetic control of immune responses. The synthetic polypeptides may be linear or branched, and possess a restricted variety of repeating antigenic determinants. For example, the synthetic polypeptide (T,G)-A–L consists of a poly-L-lysine (L) backbone carrying poly-D,L-alanine (A) branches that terminate with short random amino acid sequences of L-tyrosine and L-glutamic acid (T,G); the latter are usually the antigenic determinants (Fig. 1–1). Hapten-substituted amino acid polymers have also been used extensively, such as poly-L-lysine (PLL) to which 2,4-dinitrophenol (DNP) has been covalently linked.

HAPTENS

A hapten is a substance of low molecular weight that by itself cannot function as an immunogen but can function as a ligand. A hapten can be converted into a potent immunogen by coupling the haptenic molecule to a larger molecule termed a carrier. Autologous, allogeneic or xenogeneic protein molecules can serve as carriers. This concept is best illustrated by studies of antibody production directed against simple chemical materials, such as DNP or 2,4-dinitrofluorobenzene (DNFB), which have molecular weights of no more than a few hundred and are themselves nonimmunogenic. When coupled to large protein molecules, however, the resulting conjugate acquires immunogenic capacity and evokes an antibody response directed primarily against the simple chemical grouping which serves as the antigenic determinant.

Each one of the many haptenic groups which may be bound to the carrier protein serves as a single combining site (univalent site) on the immunogenic conjugate. Since the serologic manifestations of antigen-antibody interaction require multivalency of the antigen (see Ch. 20), a hapten in the absence of a carrier protein cannot take part in such reactions. However, the hapten is a ligand which is capable of binding to the reactive site on its corresponding antibody. Hence, the action of a hapten is best demonstrated by its ability to inhibit the reaction of its specific antibody with the complete antigen (*i.e.*, the hapten plus the carrier protein).

Recent findings regarding the T–B cell cooperative response in antibody formation (see section on Immune Response) have partially elucidated the role of the carrier protein. It appears that the haptenic sites primarily stimulate B lymphocytes which subsequently develop into cells that make antihapten antibody, while sites on the carrier protein primarily stimulate T lymphocytes to become helper cells that enhance antibody formation by the B cells. A small amount of anticarrier protein antibody is also formed so presumably some B cells react also with sites on the carrier protein. Some antibodies against hapten-carrier protein conjugates react exclusively with the haptenic group but others exhibit "carrier specificity," that is, they have a higher affin-

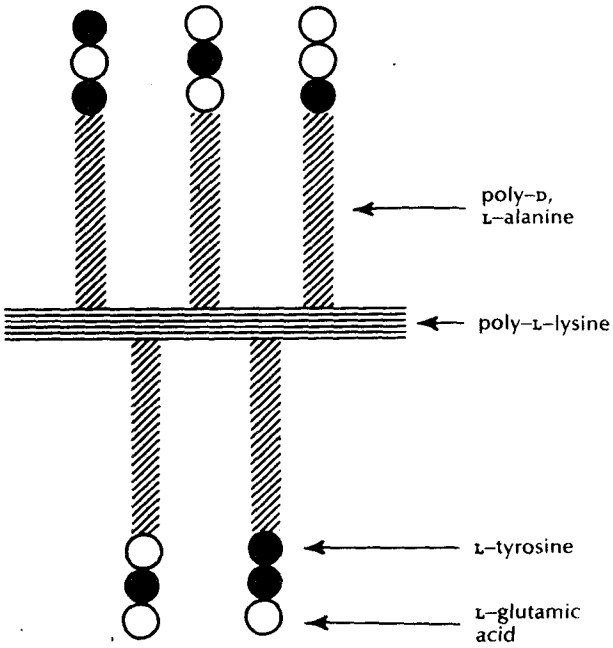

FIGURE 1–1. A multichain copolymer in which L-tyrosine and L-glutamic acid residues are attached to multi-poly-DL-alanyl—poly-L-lysine: abbreviated as poly (Tyr, Glu)-polyDLAla—poly Lys or (T,G)-A-L. (Sela M. Science 166:1365, 1969. Copyright 1969 by The American Association for the Advancement of Science)

ity for the hapten when it is attached to the same, rather than to a different, carrier. In this case, the complete antigenic combining site presumably comprises the hapten and an adjacent portion of the carrier protein.

ALLERGENS

Allergens are a special group of antigens which are innocuous to the majority of the population, but may cause disease when predisposed individuals are exposed to them by inhalation, ingestion, injection, or by contact with the skin surface. Allergens may be very simple compounds such as sulfadiazine, which probably require protein carriers during the immunogenic phase. However, the majority of other substances in the allergen class such as ragweed or grass pollens are more complex, frequently containing both protein and carbohydrate components, and having molecular weights of up to 40,000. The atopic diseases such as allergic bronchial asthma and hay fever tend to manifest themselves in individuals with a hereditary predisposition to produce IgE reaginic antibodies on exposure to environmental allergens. There is also evidence to suggest that atopic individuals may have an increased sensitivity to the chemical mediators of allergic reactions such as histamine, acetylcholine or prostaglandins (see Ch. 4).

In considering the action of allergens in atopic individuals, the portal of entry is of some importance. Individuals genetically predisposed to the development of atopic disease are usually sensitized by exposure to the allergen by way of the bronchial or gastrointestinal mucosae. Whether a specific form of interaction between the mucosal lining and the allergen or a defect in mucosal integrity, is required for this material to enter the body in an immunogenic form, is uncertain. Regardless of the mechanism, exposure of an atopic individual to the appropriate allergen results in the formation of specific antibodies of the IgE class of immunoglobulins known as reagins. On the other hand, the administration of the allergen by injection to either atopic or nonatopic individuals usually results in the formation of both IgG and IgE antibodies.

MICROBIAL ANTIGENS

The contention that a large proportion of the activity of the immune system is di-

rected against microbial antigens is based on the observation that serum immunoglobulin levels in germ-free animals are usually depressed. The antigenic composition of bacteria, viruses, fungi, and rickettsial organisms is extremely varied and complex. Potent microbial antigens may include such chemically diverse substances as the polysaccharide constituents of the pneumococcal capsule, the largely protein endotoxins of a variety of bacteria, and the primarily lipid antigens of the mycobacterial organisms. The specific antigenic determinant groups which elicit an antibody response following invasion of the body by microorganisms remain virtually unknown. Although there are a number of important exceptions, most of the antibodies formed during microbial invasion serve a protective function in the host (see Ch. 2).

TISSUE ANTIGENS

Tissue components form a group of highly immunogenic antigens. These substances include a wide variety of proteins, polysaccharides and lipoproteins that are capable of evoking either a humoral or a cell-mediated immune response. Pregnancy, passive immunization with foreign antisera and blood transfusions are the most common means by which humans are immunized to tissue components. However, the increasing frequency of human organ transplantation has provided a major stimulus for the study of the immune response following exposure to tissue antigens. This latter problem is considered in greater detail in Chapter 15.

MOLECULAR BASIS OF ANTIGENICITY

The capacity of a substance to induce antibody formation is determined by its size and its chemical nature. The lower limit at which a substance may be an immunogen is a molecular weight of approximately 1000. In general immunogenicity increases progressively with molecular size. Using synthetic and artificial antigens, it has been shown that aromatic amino acids enhance immunogenicity, especially tyrosine. Electrical charge on the other hand has no significant effect. Lipids are not antigenic in themselves.

An antigen possesses a finite number of *antigenic determinants* (epitopes, combining sites), *i.e.*, those portions of the molecule that react with the antibody or membrane receptor combining sites. For proteins, a value of approximately one determinant per 5000 MW has been estimated. The antigenic determinant has a well-defined maximal size, equivalent in volume to about 5–6 amino acid residues, with an *immunodominant* portion that makes a major contribution to its specificity. There is substantial experimental evidence that the determinants are superficially exposed on the antigen molecule. For example, a synthetic antigen rearranged so that the determinants become hidden is no longer immunogenic. The antigenic determinants of a simple polymer possess the same or a small number of different specificities, whereas those of a native globular protein express a larger variety of specificities. For each one there will be an antibody of a different specificity so that, clearly, antisera to proteins are a heterogeneous collection of many different antibody populations.

The specificity of an antigenic determinant can be *sequential* when it depends only on the particular amino acid sequence, or *conformational* when it results from the steric arrangement of the molecule due to its secondary, tertiary and even quarternary structure. Conformational determinants may therefore be composed of amino acid residues juxtaposed on the native protein but spaced apart in the unfolded polypeptide chain. For humoral responses, the majority of antigenic determinants on native globular proteins are apparently conformational, as evidenced by the relative or complete lack of cross reactivity observed between denatured proteins and antibodies to the same proteins in native form. This observation, taken together with the finding that buried determinants are not antigenic, provides strong evidence that native protein antigens are not degraded *in vivo* prior to stimulation of humoral antibody formation. Interestingly, conformational specificity is not observed correspondingly for the cell-mediated response. For example, the unfolded "loop" region of lysozyme fails to cross react with antiserum to the native antigen but cross reacts strongly when tested by delayed-type cutaneous hypersensitivity and by *in vitro* lymphocyte transformation. Thus, T cell-mediated recognition appears to depend on sequential rather than conformational determinants and seems to have less stringent specificity requirements than for B cell recognition. (The cellular basis for cell-mediated and humoral immune responses is discussed in the section on Immune Response).

An antigen is not necessarily equally immunogenic with respect to evoking

humoral and cell-mediated responses. In fact, there is evidence that an inverse relationship exists. This is a form of "immune deviation" (see section on Immunologic Unresponsiveness). For example, chemical modification of the bacterial protein flagellin or of sheep erythrocytes has been shown to evoke a lower antibody response than that obtained with the unmodified antigen, yet it induces much higher levels of delayed-type hypersensitivity. Similarly, denatured proteins preferentially induce cell-mediated immunity. The mechanism underlying this selective stimulation of one type of immunity is unknown but presumably operates at the level of antigen recognition by T and B cells.

IMMUNOGLOBIN STRUCTURE AND SYNTHESIS

Serum proteins separated by various electrophoretic techniques were originally designated as albumin, α1-, α2-, β- and γ-globulins. The term immunoglobulin (Ig), as distinct from the term gammaglobulin, is commonly used in a more functional context to describe serum proteins with antibody characteristics that include the γ-globulins as well as serum proteins that extend into the β and α2 range of electrophoretic mobility (Fig. 1–2).

STRUCTURE OF IMMUNOGLOBULINS

The core or monomeric unit structure of immunoglobulin (Ig) molecules consists of four polypeptide chains—two identical heavy (H) and two identical light (L) chains—and can be represented by the molecular formula H_2L_2. In each molecule, the H and L chains are held together by disulfide bonds and by noncovalent hydrophobic interactions. Superimposed upon this basic prototype four-chain structure are differences in size, carbohydrate content, biologic activity and antigenicity (Tables 1–1 and 1–2). These differences, found primarily on the heavy chains, can be used to identify five major classes of immunoglobulins—IgG, IgA, IgM, IgD and IgE. The Greek letters, γ, μ, α, δ and ε refer to the heavy

FIGURE 1–2. Electrophoretic migration of human immunoglobulins.

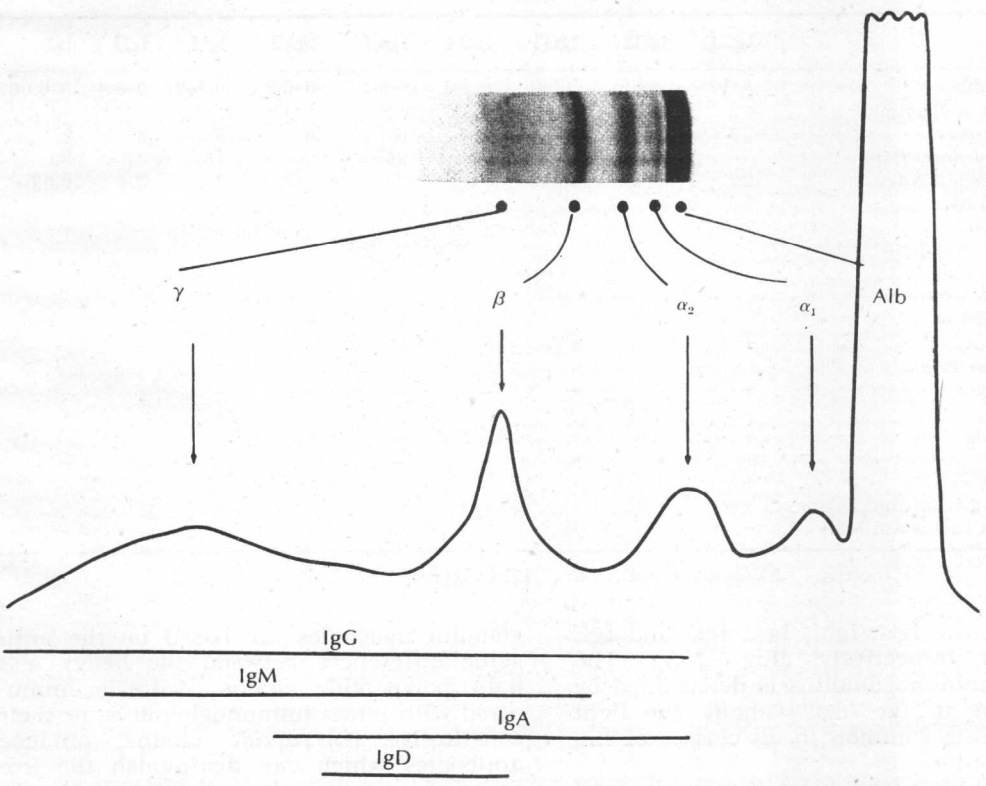

TABLE 1–1. Classes of Human Immunoglobins

Characteristics	Class				
	IgG	IgA	IgM	IgD	IgE
Molecular formula	$(\kappa_2\gamma_2)$ or $(\lambda_2\gamma_2)$	$(\kappa_2\alpha_2)_n$† or $(\lambda_2\alpha_2)_n$	$(\kappa_2\mu_2)_5$ or $(\lambda_2\mu_2)_5$	$(\kappa_2\delta_2)$ or $(\lambda_2\delta_2)$	$(\kappa_2\epsilon_2)$ or $(\lambda_2\epsilon_2)$
Molecular weight	150,000	$(160,000)_n$	900,000	180,000	200,000
Heavy Chains					
Class	γ	α	μ	δ	ϵ
Subclasses	γ1,2,3,4	α1,2	μ1,2	–	–
Molecular weight	53,000	58,000	70,000	65,000	72,000
Allotypes	Gm	Am	–	–	–
Light Chains					
Type	κ,λ	κ,λ	κ,λ	κ,λ	κ,λ
Molecular weight	22,500	22,500	22,500	22,500	22,500
Allotypes	Inv	Inv	Inv	Inv	Inv
J Chain	–	+	+	–	–
Secretory piece	–	+	+*	–	–
Carbohydrate (%)	3	7	12	13	11
Antigen binding sites	2	2	5–10	2	2
Serum level (mg/100 ml)	600–1800	200–500	60–200	0.1–4.0	0.01–0.9

*Found on secretory IgM
†n = 1–3

TABLE 1–2. Biologic Properties of Immunoglobins

Property	IgG1	IgG2	IgG3	IgG4	IgA1	IgA2	IgM	IgD	IgE
Serum (mg/ml)	5–12	2–6	0.5–1	0.2–1	0.5–2	0–0.2	0.5–2	0–0.4	0–0.002
Distribution in secretions	–	–	–	–	+++	+++	++	–	+
Half Life in days	23	23	16	23	6	6	5	3	2
Fractional turnover (%)	7	7	17	7	25		18	37	89
Synthesis: mgm/kg/day	25		3.4		24		7	0.4	0.02
Placental transfer	+	+	+	+	–	–	–	–	–
P–K Reactivity	–	–	–	–	–	–	–	–	+
Complement fixation: Classic pathway	+++	+	+++	–	–	–	+++	–	–
Complement fixation: alternate pathway	–	–	–		+	+	–	+	+
Reverse P.C.A.	+	–	+	+	–	–	–	–	–
Receptors for Fc on:									
Macrophages	+	–	+	–	–	–	–	–	–
Basophils	–	–	–	–	–	–	–	–	+
Neutrophils	+	–	+	–	–	–	–	–	–
Platelets	+	+	+	+	–	–	–	–	–
Lymphocytes	+	?	+	?	–	–	–	–	–
Antigen for Rheumatoid Factor	+++	+++	–	+++	+		+		+
Rheumatoid Factor Antibody	+	+	+	+	+	+	+++	–	–

(+) Positive (–) Negative Blank space, not tested (?) Uncertain

chains of the IgG, IgM, IgA, IgD and IgE molecules respectively (Fig. 1–3). The class of immunoglobulins is determined by the nature of the heavy chain, the light chains being common to all classes of immunoglobulin.

The simplest and most commonly used methods for identifying individual immunoglobulin molecules are based on the antigenic differences between the heavy and light polypeptide chains. Animals immunized with intact immunoglobulins, or their constitutive polypeptide chains, produce antibodies which can distinguish the five types of immunoglobulin heavy chains. In addition, antisera have been produced that

have shown that the light chains consist of two major antigenic types, called kappa (κ) and lambda (λ). The tetrapolypeptide chain structure of the monomeric units of each immunoglobulin class is composed of a pair of identical light chains of the kappa or lambda type and not a hybrid mixture of the two.

Considerable insight into the structure of immunoglobulin molecules has been obtained through the study of monoclonal myeloma and macroglobulinemia proteins. These proteins are structurally similar to circulatory Ig and represent the homogeneous products of single clones of neoplastic plasma cells. Some plasma cell tumors were found to synthesize a low molecular weight product called Bence-Jones protein which is readily excreted in the urine. Bence–Jones proteins were shown to possess unusual thermal properties. They precipitate on heating to 45–60°C, redissolve on boiling, and reprecipitate on cooling. Most other proteins coagulate irreversibly on boiling. It was subsequently demonstrated that Bence-Jones proteins are identical to light chains. Since the products of plasma cell tumors represent homogeneous molecules derived from a single clone of cells that can be isolated in pure form in large quantities from either serum or urine, they have been widely used in amino acid sequence analysis, and in structural and genetic studies of the immunoglobulins. The immunoglobulins normally found in the circulation are heterogeneous, and represent the sum of the products of thousands of clones of different antibody forming cells.

INTERACTION BETWEEN HEAVY AND LIGHT CHAINS

Disulfide bridges constitute a prominent part of the structure of immunoglobulin molecules. Interchain disulfide bonds (–S–S–) are found between the H and L chains and between the two H chains of the monomeric unit of all immunoglobulin molecules; they are not required to maintain the integrity of immunoglobulin molecules. They may be split by reduction, and their reformation may then be prevented by subsequent alkylation. In this fashion, and by subsequent molecular sieve chromatography in dissociating agents, the individual H and L chains can be separated. The isolated chains of IgG molecules can be recombined and antigen binding restored, indicating that noncovalent, hydrophobic interactions are primarily responsible for maintaining the structure of these molecules.

The interchain disulfide bonds differ both in number and position in the different classes and subclasses of heavy chains, and in the two light chain types. The formation of an inter-heavy-light chain disulfide bond involves a cysteine that is C-terminal in K chains, while in λ chains the cysteine is penultimate to the carboxy terminus. In all immunoglobulin molecules, the corresponding cysteine in the heavy chain is found closer to the amino terminal end of this polypeptide chain (about position 131). The exceptions to this rule are the IgG1 subclass, where the cysteine is about 100 residues closer to the carboxy terminal end of the H chain, and the molecules of the IgA2 subclass that do not contain an inter-heavy-light disulfide bond. The inter-heavy chain disulfide bonds are found in the hinge region of these molecules, where they show some variation within the immunoglobulin subclasses (Fig. 1–3).

In contrast to the variation in position of the interchain disulfide bonds, intrachain disulfide bonds occur with virtual constancy throughout both the H and L polypeptide chains. These intrachain disulfide bridges are conserved throughout evolution in all the immunoglobulin classes, suggesting they are important in maintaining the overall conformation of these molecules.

CLASSES OF IMMUNOGLOBULINS

IgG

These molecules constitute over 85% of the total circulating antibody molecules. Hydrodynamic studies, subsequently confirmed by electron microscopy and x-ray crystallography have revealed that these molecules have a Y shape (Fig. 1–4). Considerable insight into the biologic activity of immunoglobulins has been obtained by proteolytic enzyme cleavage of the IgG molecule. Hydrolysis of the IgG molecule with the proteolytic enzymes trypsin, papain, and pepsin produces characteristic products (see Fig. 1–3). Papain and trypsin attack the heavy chain at the N or amino terminal side of the interheavy chain disulfide bonds, liberating the Fc fragment and two Fab fragments. The Fc fragment is a dimer of the C- or carboxy-terminal portion of the heavy chain, whereas the two Fab fragments consist of an intact light chain and an amino-terminal half or Fd fragment of the heavy chain linked together by a single H–L inter-

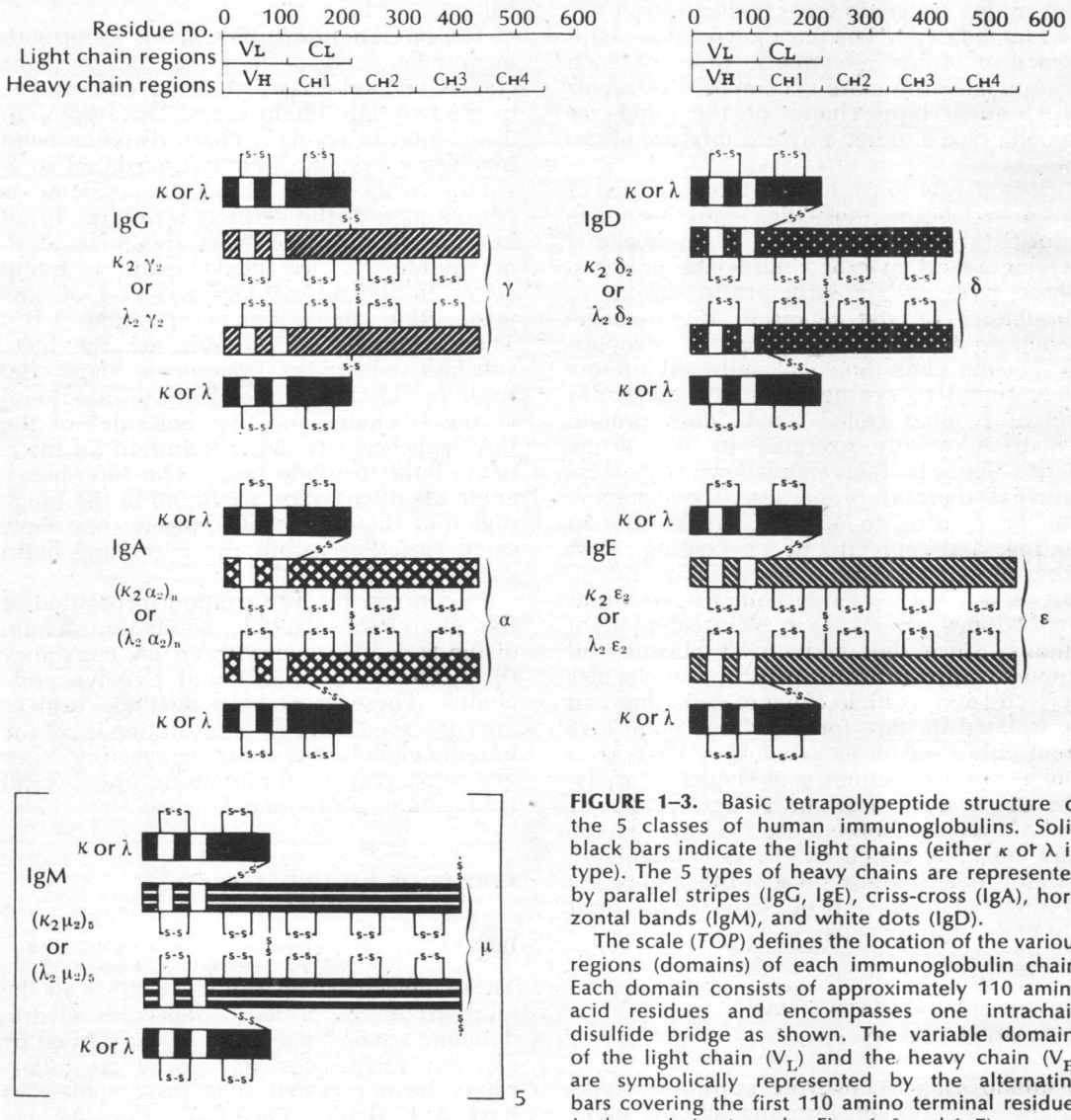

FIGURE 1–3. Basic tetrapolypeptide structure of the 5 classes of human immunoglobulins. Solid black bars indicate the light chains (either κ or λ in type). The 5 types of heavy chains are represented by parallel stripes (IgG, IgE), criss-cross (IgA), horizontal bands (IgM), and white dots (IgD).

The scale (*TOP*) defines the location of the various regions (domains) of each immunoglobulin chain. Each domain consists of approximately 110 amino acid residues and encompasses one intrachain disulfide bridge as shown. The variable domains of the light chain (V_L) and the heavy chain (V_H) are symbolically represented by the alternating bars covering the first 110 amino terminal residues in those chains (see also Figs. 1–6 and 1–7).

chain disulfide bond. It is the Fab fragments that contain the antigen-combining sites of the antibody molecules; hence, the IgG immunologobulin molecule is divalent. The Fc fragment, containing most of the carbohydrate of the IgG molecule, has a tendency to crystallize, but does not have the capacity to combine with antigen. Pepsin, unlike papain or trypsin, hydrolyzes the immunoglobulin molecule on the C-terminal side of the inter-heavy chain disulfide bonds, liberating a single bivalent antibody fragment called $F(ab')_2$. The fragment has a molecular weight of 110,000.

Studies of the biologic properties of Fab and Fc fragments have shown that the immunoglobulin molecule can be divided into two distinct functional regions. The region of the molecule encompassing the Fab fragment is responsible for specific antigen binding while the Fc portion of the heavy chain determines the effector functions of antibody molecules such as complement-fixation, transplacental passage and binding to mast cells (Table 1–2). The two Fab fragments are separated from the Fc fragment by the so-called hinge region. This region contains a large number of proline residues and a varying number of cysteine residues that are involved in the disulfide bridges linking the two heavy chains. Pre-