Volume I

CLINICAL IMMUNOLOGY

Edited by

CHARLES W. PARKER, M.D.

1980 W.B. Saunders Company
Philadelphia London Toronto

CLINICAL IMMUNOLOGY

Volume I

Edited by

CHARLES W. PARKER, M.D.

Professor of Medicine and
Chief, Division of Allergy and Immunology
Department of Medicine, and
Professor of Microbiology and Immunology
Washington University School of Medicine
St. Louis, Missouri
Investigator, Howard Hughes Medical Institute

CONTENTS

VOLUME I

VOLUME II

Chapter 1

STRUCTURE AND FUNCTION OF IMMUNOGLOBULINS

DAVID W. ANDREWS*
AND J. DONALD CAPRA

A goal of modern medicine is the attempt to explain clinical phenomena in molecular terms. In clinical immunology, these explanations generally revolve around specific antibody molecules and/or the cells of the lymphocyte series which bear specific receptors on their surfaces. In concrete terms, much more is known about antibody molecules than about either the cells that produce them or other cells that mediate immunologic phenomena. Because of ease of availability of immunoglobulin molecules, they have become the prototypic specificity molecule, not only for immunology but for molecular biology as well.

The immunoglobulins comprise a set of glycoproteins, peculiar to vertebrates, which are distributed both intra- and extravascularly and in mucous secretions. Their function is the recognition and specific binding of antigenic determinants (patterns, epitopes, combining sites) displayed on other molecules. The production of immunoglobulins, which are the secretory products of plasma cells derived from B lymphocytes, is elicited by the interaction of lymphocytes with previously unencountered antigens in the appropriate milieu. The immunoglobulins produced in such a response represent a heterogeneous population of molecules, differing in average molecular charge and affinity for the immunogen. They share a common structural motif, yet the product of each clone of lymphocytes is itself unique. The mechanism of the generation of sufficient diversity in this recognition system, so that an individual may respond to a wide variety of environmental antigens, is a major mystery. The extent of this diversity (i.e., how many unique combining sites exist) is unknown but must be very large.

HISTORICAL PERSPECTIVE

The classic criteria for characterization of serum proteins are size, solubility, and ionic character. Unfortunately, none of these parameters defines uniquely the immuno-

*DWA is supported by a training grant (CA 09082) awarded by The National Cancer Institute to the Graduate Program in Immunology.

globulins. Their heterogeneity in structure would have been a nearly insurmountable barrier were it not for the early appreciation that myeloma proteins were valid representatives of these molecules. A vast literature (Kunkel et al., 1963; Grey and Kunkel, 1964; Kunkel and Prendergast, 1966) supports the view that a small percentage of lymphoid malignancies spontaneously produce large amounts of homogeneous products indistinguishable from "normal" immunoglobulin molecules. The use of the protein products of this spontaneous disease in humans, as well as in dogs (Capra and Hurvitz, 1970), cats (Kehoe and Capra, 1972), and rats (Bazin et al., 1972), and its induction in mice (Potter and Boyce, 1962) has been crucial in the definition of the antigenic, structural, functional, and evolutional attributes of immunoglobulins.

The isolation of homogeneous induced antibodies has generally been precluded by the heterogeneous response to most immunogens alluded to previously. In some cases, however, the production of a homogeneous response has been achieved (Krause, 1970). While rare and often transient in nature, the production of these molecules is extremely useful because they have been induced by a structurally defined immunogen. This important feature is obviously lacking in myeloma proteins, although diligent searches occasionally identify antigens for which these molecules have affinity (Metzger, 1969). Interestingly, Seligmann has recently reported a human myeloma protein with high affinity for horse gamma globulin in a man who had been injected with horse antitoxin 20 years previously.

Serum proteins can be separated according to their buoyant density (an indirect, but useful, correlate of their size) in the ultracentrifuge; three major fractions are observed, in order of decreasing size, called 19S, 7S, and 4S. Immunoglobulins can be demonstrated in both the 19S and 7S fractions. Heidelberger and Pederson (1937) were the first to separate immunoglobulins by size when they showed that horse antipneumococcal antibodies could be assigned to fractions corresponding to a molecular weight of 900/kdal (the 19S fraction as above) and 150/kdal (7S). The larger fraction was named "immunoglobulin macro," or IgM, and the smaller "immunoglobulin gamma," or IgG, a reflection of its electrophoretic mobility. Both designations persist to the present.

Elvin Kabat, working in the laboratory of the Swedish chemist Arne Tiselius, made some critical observations concerning the ionic character of elicited antibodies (Tiselius and Kabat, 1939). He performed electrophoresis on the sera of rabbits intensively immunized with ovalbumin and compared the patterns produced with those derived from the sera of normal, unimmunized rabbits (Fig. 1–1); there was a clear enhancement of the gamma globulin fraction in the

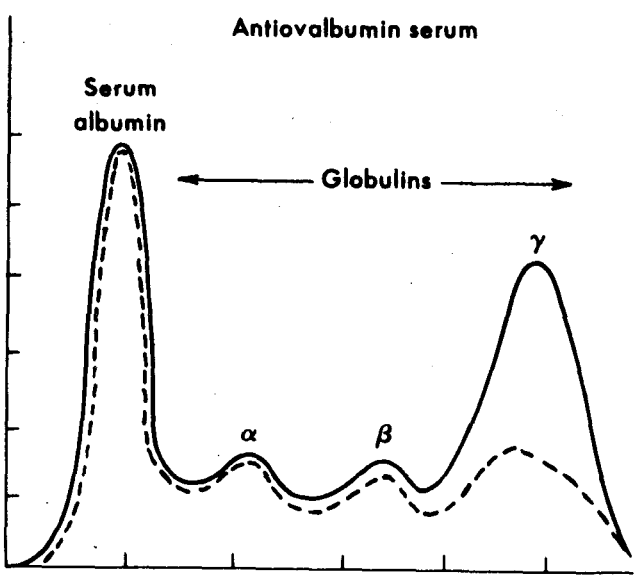

Figure 1–1. Electrophoresis of sera from rabbits immunized with ovalbumin, before (———) and after (·····) precipitation with the immunogen.

immunized animals. When these sera were mixed with ovalbumin, a precipitate was formed, and electrophoresis of the supernatant revealed a depletion in this same fraction. This conclusion is reflected in the use of the terms *gamma globulin* and *immunoglobulin* as synonyms, although immunoglobulins are present to a lesser extent in other electrophoretic fractions. Eisen and Karush (1949) purified 7S antibody specific for the *p*-azobenzenearsonate hapten and showed that each molecule of antibody had two binding sites for hapten, explaining the formation of precipitates with multivalent antigens.

The elucidation of the basic topography of the immunoglobulin molecule led to Nobel prizes for Rodney Porter and Gerald Edelman. Porter, working in London, utilized antibodies prepared from the sera of rabbits immunized with ovalbumin. Using the restricted specificity of the enzyme papain and the resistant nature of the globular structure of the native molecules, he was able to cleave these immunoglobulins into two fragments, separable by ion-exchange chromatography (Porter, 1959). One fraction retained the capability to react with the immunogen and was called the antigen-binding fraction, or Fab. The other crystallized upon standing and was called the crystallizable fraction, or Fc. Since the Fab fragment bound, but did not precipitate, with the immunogen, Porter concluded that it was univalent. Further, the Fab fragments were produced in twice the yield of Fc, and their molecular weights were essentially the same. This confirmed the earlier evidence that antibodies were divalent. The Fc portion of the molecule was later shown to be necessary for a number of the effector functions of the immunoglobulin molecule, including complement activation, cell binding, and placental transport. It was further shown that when pepsin instead of papain was used for digestion, the two Fab portions of the molecule remained attached to one another through a single disulfide bond [$F(ab')_2$ fragments].

Edelman, working at the Rockefeller Institute, performed a complete reduction and alkylation on a gamma globulin pool (Edelman and Poulik, 1961). This procedure was designed to separate disulfide-bonded polypeptide chains. When the reaction mixture was exposed to urea, a strongly denaturing solvent, two fractions could be resolved according to size, and in equivalent quantities. The larger, having a molecular weight of 50 kdal, was called "heavy chain," and the smaller "light chain" was of 25 kdal. Clearly, two of each type of chain could constitute a native immunoglobulin.

Later studies in Porter's laboratory (Fleischman et al., 1963) delineated the relationship between the chain structure and the proteolytic fragments, and a general model for immunoglobulin structure was proposed. Although modified by more recent information, the model (Fig. 1-2) is representative of these original deductions and a tribute to the perspicacity of these

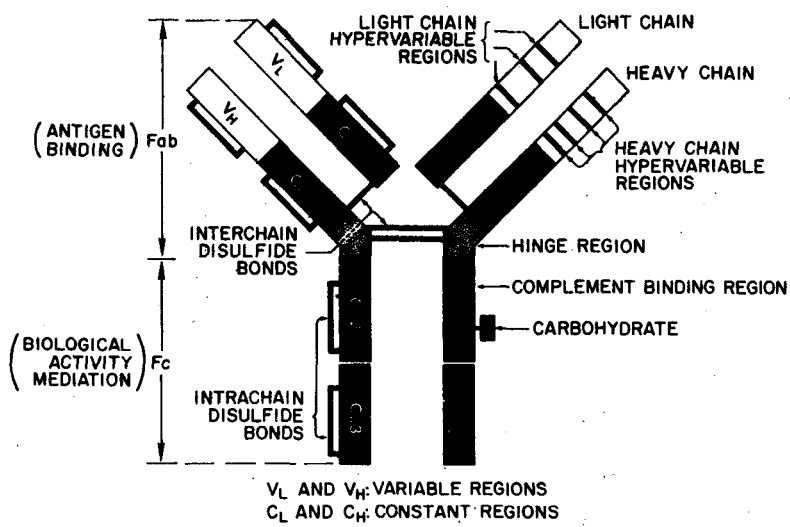

Figure 1-2. Model of the prototypical immunoglobulin molecule.

workers. This structure, containing the two heavy chain, two light chain (H2L2) configuration, is the prototype for all immunoglobulins.

Another useful approach in characterizing immunoglobulins is antigenic analysis. The use of this route stems from the fact that immunoglobulins are themselves highly immunogenic when used as antigens in disparate species. Such heterologous antisera are usually elicited by injection of whole serum and can be used to detect the major classes in humans — IgG, IgA, and IgM. This is most graphically demonstrated using the technique of immunoelectrophoresis. Two less abundant but antigenically distinct immunoglobulins, IgD and IgE, are not detected by this method, owing primarily to their low concentration in serum, although they can now be measured by more sensitive immunochemical methods. These two immunoglobulin classes were defined when myeloma proteins emerged that were unreactive to conventional anti-immunoglobulin antisera, although IgE immunoglobulins had already been partially characterized by immunization with IgE antibodies from allergic patients.

Careful and more discriminating antigen analysis revealed the presence of further categories, or subclasses, in IgG and IgA and possibly in IgM and IgD. These subtle differences, as well as those observed among the classes, are determined by differences in sequence among the respective heavy chains. The heavy chain of a particular class of immunoglobulin is designated as the Greek letter corresponding to its class. Thus, IgG possesses γ (gamma) heavy chains, IgM has μ (mu), IgA has α (alpha), IgD has δ (delta), and IgE has ϵ (epsilon).

Patients afflicted with multiple myeloma frequently excrete in their urine a protein sharing some antigenic determinants with other myeloma proteins. These proteins can be divided into two types, called kappa (κ) and lambda (λ), and have been identified as light chains or their dimers or fragments. These two light chain types are represented in all of the immunoglobulin classes. A given immunoglobulin molecule has κ chains or λ chains but not both. In man, the normal ratio of κ- to λ-containing immunoglobulins is two to one, but in antibodies with a given specificity this ratio is frequently altered, suggesting that one or the other light chain type may be better adapted to making antibodies to that particular antigen.

Thus, the complete description of a particular myeloma protein or immunoglobulin requires the designation of both heavy chain class and subclass, and light chain type, e.g., IgG3κ.

PRIMARY STRUCTURE

The development of amino acid sequence analysis of proteins ushered in an era of unrestrained data production in all areas of biology which is only now beginning to abate. In immunology, the seminal work of Hilschmann and Craig (1965) stands out. These workers set out to sequence completely two κ myeloma light chains, purposefully chosen to be serologically disparate. The result of this undertaking was the observation that the amino terminal halves of the two chains were quite different, but the carboxy terminal halves were virtually identical! This unexpected result prompted the authors to promote genetic heresy; they proposed that two structural genes must be involved in the production of this single polypeptide chain. The reality of this insightful proposal has been fervently debated and has only recently been confirmed, ironically, by DNA sequencing (Brach and Tonegawa, 1977). Another deduction from these and other data was that the capacity to recognize and bind antigen lay in the variable, amino terminal portion of these molecules.

The complete sequence of an immunoglobulin molecule was accomplished by Edelman et al. (1969), once again using a myeloma protein. The light chain was 224 residues long and homologous to those sequenced by Hilschmann and Craig. The nature of the heavy chain sequence was such that its 446 amino acids could be divided into four homology units of about 110 residues, each of which was stabilized by an intrachain disulfide bond. This characteristic prompted the proposal (Edelman and Gall, 1969) that each unit formed a "domain" and that each domain of the heavy and light chain had evolved to fulfill a particular biologic function. The most obvious example was the antigen-binding function of the light and heavy chain variable regions.

Critical examination of extensive se-

quence data revealed the unique nature of immunoglobulins. With the exception of minor genetic polymorphisms, variability in light chains seemed to be confined to the amino terminal half, the carboxy terminus being constant for a given type (Hilschmann and Craig, 1965; Titani et al., 1965; Milstein, 1966; Putnam et al., 1966). The same is true in heavy chains (Capra and Kehoe, 1974a), with the amino terminal domain being variable with respect to other heavy chains, while the remaining domains are constant for a particular class and subclass. Thus, the terms variable (V) and constant (C) region arose, with V_L and C_L signifying the domains of the light chain, and V_H and C_H those of heavy chains. The constant region of the heavy chain could be further divided into homology units, as alluded to previously, of similar but not identical sequence. These were numbered consecutively from the amino terminus of the chain C_H1, C_H2, C_H3 and, in the μ and ϵ chains, C_H4. The presence of a section of varying length, containing the cysteines involved in heavy-heavy disulfide bonds and also rich in proline residues, was noted in sequences of some heavy chain classes between the Fc and Fab portions. This section, descriptively called the "hinge," is depicted in Figure 1–2. Indeed, the Fab portions of antibody molecules can apparently pivot about this hinge to a remarkable degree (Werner et al., 1972; Cathou and O'Konski, 1970; Valentine and Green, 1967).

Using enzymatically and chemically derived fragments of heavy chains, the functions of complement fixation and binding to macrophages have been assigned to the $C_\gamma2$ (Kehoe et al., 1969; Dorrington and Painter, 1974) and $C_\gamma3$ (Yasmeen et al., 1973) domains, respectively.

The extent of variability among a variety of light and heavy chain variable regions is subject to certain constraints. Characteristic segments are conserved from one V_H or V_L to another, particularly those near to and including the cysteine residues responsible for the intrachain disulfide bonds. Similar stretches occur in V_H regions. In human V_H sequences, 14 per cent of the residues are absolutely invariant, while a substantial number of other positions exhibit limited variability (Capra and Kehoe, 1974b).

Similarly, restricted and noncontiguous portions of both heavy and light chain variable regions manifest profound variability (Fig. 1–3), such that these regions have been called "hypervariable." This distinction between relatively conserved and hypervariable portions of variable domains has been interpreted to mean that the conserved segments serve as a framework in which the hypervariable segments may fold together to form the antigen-binding site.

Another approach to the antigen-binding site was through the use of affinity labeling. This technique had been applied to enzymes and seemed admirably suited to probing the "active sites" of antibodies. Briefly, the technique involves reaction of an antibody with an antigen, modified so as to be chemically reactive. For example, bromoacetyl groups and diazonium salts were frequently utilized to induce the formation of covalent bonds between antibody and antigen. These reactions were necessarily restricted to nucleophilic or aromatic amino acids and, as such, lacked strict specificity for residues within the combining site. Other studies (Press et al., 1971; Fleet et al., 1969, 1972) involved using antigens modified with photochemically labile moieties, such as the azido group. These antigens were allowed to bind their antibodies, and then were converted to their reactive intermediates by light of the appropriate wavelength. This reaction produced a "nitrene," capable of reacting with virtually any amino acid, thus obviating the specificity objection raised earlier.

After the affinity labeling reaction, the heavy and light chains are separated and subjected to enzymatic digestion. The peptides covalently bound to the antigen can be identified, sequenced, and located within the molecule.

Using these methods, an impressive body of information has accumulated concerning the residues involved in antigen binding (Fig. 1–3). Affinity labels were identified in the hypervariable regions of light (Goetzl and Metzger, 1970; Franek, 1971; Chesebro and Metzger, 1972) and heavy chains (Ray and Cebra, 1972; Haimovich et al., 1972; Press et al., 1971), adding additional inferential evidence that those regions constitute the antibody-combining site. A logical inference from the above discussion is that both H and L chains are present in the antibody-combining site, with each contributing to antigen binding. In accord with this possibility, when H and L chains are separated from one another they bind weakly or

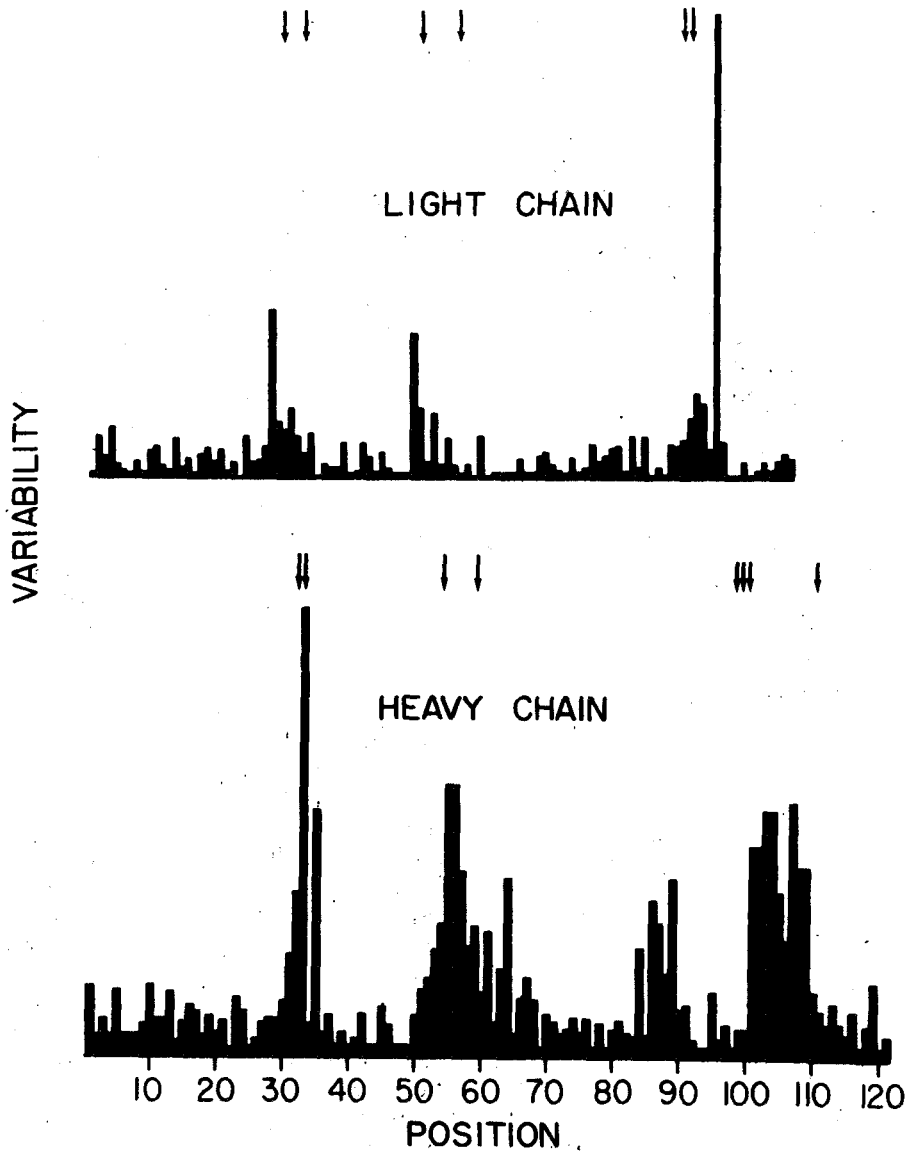

Figure 1–3. Plots of variability in light and heavy chains according to the method of Wu and Kabat (1970). Localization of affinity-labeled residues is indicated by arrows.

not at all to antigen. When recombination experiments are performed with the original H and L chains binding is restored, but if either the H or the L chain is from an antibody to an unrelated antigen little or no restoration is obtained.

All of the immunoglobulins are glycoproteins with a carbohydrate content varying from 3 per cent (IgG) to 12 per cent (IgM). The IgG subclasses have a single oligosaccharide chain attached to a single asparagine residue in the $C_\gamma 2$ domain of the H chain. The other immunoglobulins have several oligosaccharide chains per heavy chain.

GENERAL FEATURES AND FUNCTIONS OF IMMUNOGLOBULINS

The various immunoglobulins differ in valence (IgM and polymeric IgA have a higher valence than the other immunoglobulins), ability to fix complement, half-life, body distribution, affinity for cells, and time

and control of biosynthesis, indicating that they subserve specialized functions in the immune recognition system. However, as stated in the opening paragraph, they have a common function, which is one of antigen recognition. The perception and subsequent binding of such patterns can have a number of consequences:

Direct Neutralization of Antigen

In the case of "foreign" molecules or organisms, such recognition can lead to inactivation (e.g., viral and toxin neutralization) due to actual physical masking of receptor sites needed for effective penetration into or interaction with tissues.

Promotion of an Inflammatory Response

Due to interactions with complement, chemotactic cleavage products are released which lead to the formation of a cellular exudate (see Chapters 7, 8, and 9). The clotting and kallikrein systems may also be activated. IgE antibodies attached to mast cells release mediators of immediate hypersensitivity and other chemotactic factors.

Stimulation of Antigen Destruction or Disposal

Complement and attached immunoglobulins in immune complexes stimulate antigen disposal and destruction by promoting phagocytosis, lysosomal enzyme release, and other activities in neutrophils and monocytes. Cell-bound immunoglobulins are responsible for some forms of lymphocyte-mediated cytotoxicity (see Chapter 7).

Modulation of the Immune Response

Through their effects on antigen disposal or through direct actions of antigen-antibody complexes on T and B lymphocytes, antibodies exert a regulatory function in the immune response. Indeed, the antigenic determinants displayed by the combining sites of immunoglobulins (idiotypes — see below) have been implicated in a regulatory network spanning the entire immune response, including the cellular responses of both B and T cells (Jerne, 1974) (see Chapter 4).

The recognition of antigenic groupings by antibodies can lead to unhappy outcomes as well. Tumor antigens can be bound by antibodies, preventing the destruction of cells bearing these antigens by cellular components of the immune response (see Chapter 13). Binding of low-affinity antibodies to bacterial antigens to form nonprecipitating complexes can lead to glomerulonephritis when these complexes deposit in the kidney (see Chapter 33). Antierythrocyte antibodies of the appropriate class can traverse the placenta and place an incompatible fetus in a life-threatening situation (see Chapters 30 and 31). Finally, the production of "autoantibodies" seems to underlie a wide spectrum of human disease states (see Chapter 4). In some instances these may represent immune responses to foreign antigens that fortuitously cross-react with autoantigens, but in many instances it seems highly likely that the tissue antigens themselves stimulate a response.

CLASSES OF IMMUNOGLOBULIN

IgG

IgG is the most abundant of the classes of immunoglobulins, its concentration in serum being around 1200 mg/100 ml. The

TABLE 1-1. PROPERTIES OF THE SUBCLASSES OF HUMAN IgG

PROPERTY	IgG1	IgG2	IgG3	IgG4
Serum concentration (mg/ml)	5–12	2–6	0.5–1.5	0.1–0.8
Half-life (in days)	23	23	11	21
Placental transfer	+	+	+	+
Cl_q binding	++++	++	++++	+/−
Skin fixation (reverse PCA)	+	−	+	+
Monocyte receptor	+	−	+	−
PMN receptor	+	−	+	−
Reactivity with staphylococcal protein A	+	+	−	+

structural definition of this class has already been discussed, although the complete sequence of only one of the four subclasses is available. The various properties of these subclasses are listed in Table 1–1. IgG1 has the highest concentration in human serum, followed in order by IgG2, IgG3, and IgG4.

While it is clear that the individual IgG subclasses are under separate genetic control, they clearly are related more closely to one another than to the members of other immunoglobulin classes. The differences in function are actually striking in view of the comparative sequence studies. All the subclasses are bivalent and exhibit 95 per cent amino acid sequence homology, the majority of differences residing in the hinge region. The IgG subclass with the greatest structural disparity is IgG3, which has an extended hinge region containing more than 100 amino acid residues, giving it a molecular weight about 10,000 daltons higher than the other IgG subclasses. This structural alteration apparently explains why IgG3 is more susceptible to proteolytic degradation and has a shorter biologic half-life *in vivo*. IgG1 and IgG3 interact more readily with complement than do IgG2 and IgG4 and also are more cytophilic, binding to monocytes, neutrophils, and lymphocytes even when unaggregated. The other IgG subclasses have to be aggregated in order to interact effectively with these cells. IgG1, IgG2, and IgG4 interact with a protein present in staphylococcal cell walls (protein A), but whether or not this interaction represents a form of neutral resistance to staphylococci remains to be established.

As a rule, immunization is associated with an antibody response in all four subclasses, although not infrequently selectivity is demonstrated, as for example with carbohydrate antigens that produce primarily an IgG2 response. IgG antibody responses are especially rapid and marked during secondary immune responses, often accounting for 90 per cent or more of the antigen-binding activity in serum after multiple exposure to antigen. However, there is a limited IgG antibody response toward the end of the first week of immunization. While small amounts of IgG antibody are synthesized by the fetus, most of the IgG antibody present at birth is obtained by placental transfer from the mother. All four IgG subclasses cross the placenta, whereas the other immunoglobulin classes do so to a very limited extent or not at all. In some animal species significant amounts of the immunoglobulin in colostrum are absorbed via the gastrointestinal tract, particularly during the first several days after birth. In man, absorption of antibody from the intestine occurs, but the quantities involved are small.

IgA

IgA is the next most abundant immunoglobulin in serum but is the predominant immunoglobulin in mucous secretions (e.g., oral, urogenital, nasal, bronchial, and intestinal secretions, tears, milk). Congenital deficiency of IgA is one of the most common genetic disorders in humans (1 in 600 children) and almost certainly involves a defect in regulation of immunoglobulin production. A single instance of secretory component deficit (see below) has been described (Strober et al., 1976).

IgA synthesis is virtually undetectable in the fetus and does not become substantial for several months after birth. Adult levels are not reached until age 5 years. Most of the IgA in exocrine secretions appears to be locally synthesized in plasma cells in the submucosa or regional nodes. The gut (and presumably other mucosal surfaces) contains specialized populations of IgA-producing cells which undergo local stimulation by antigen and then in part redistribute themselves to other mucosal areas. This is indicated in a recent study in which immunization by ingestion of capsules containing cariogenic streptococcal antigens resulted in increased levels of antibody in saliva.

The degree of polymerization of serum IgA varies markedly among species and even among individuals within a species. In humans, the primary form of serum IgA is the H2L2 monomer, but polymeric forms of up to 18S can be detected (Tomasi, 1972). Theoretically, polymerization should increase the effective valence of the IgA and therefore its biologic effectiveness.

In secretions, by far the predominant form is the dimer (Fig. 1–4), which contains, in addition to the two H2L2 monomers, a J chain and a glycopeptide called secretory component (Tomasi, 1972). Thus, dimeric IgA consists of a combination of four unique polypeptide chains: heavy and light immunoglobulin chains, J chain, and secretory component (SC). These subunits are

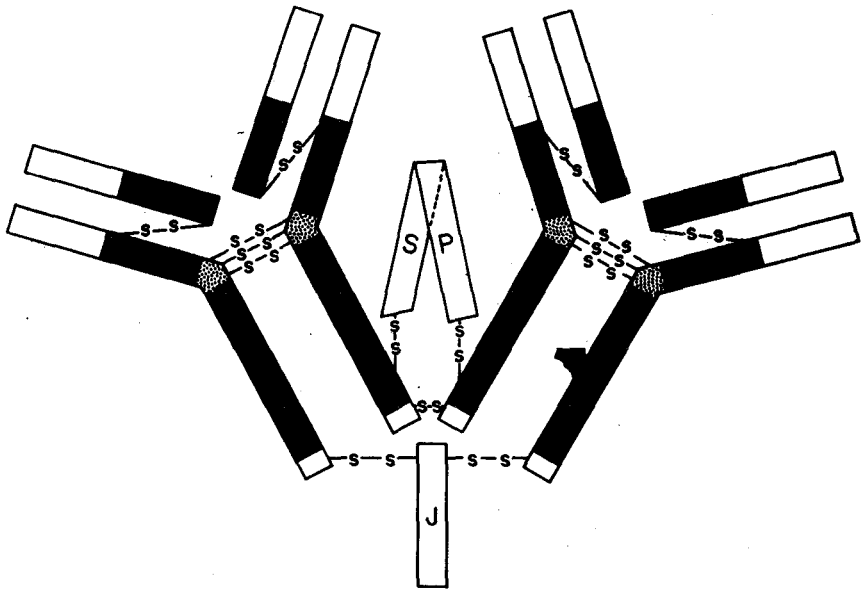

Figure 1–4. Schematic representation of human secretory IgA. Four unique polypeptide chains are indicated: heavy and light immunoglobulin chains, J-chain, and secretory component.

linked with one another by disulfide bonds as well as by noncovalent interactions.

The J (for "joining") chain has a molecular weight of 15 kdal (Wilde and Koshland, 1973) and appears to be necessary for the proper assembly of dimeric IgA. All but SC are synthesized in the lymphocyte; SC is added after release of the dimer, during traversal of the epithelium. The function of SC, a chain of 60 kdal molecular weight, is unknown, but it may be involved in the extrusion of the completed sIgA molecule into the extracellular space, or its protection from proteolytic degradation after secretion. A possible role in secretion is suggested by the fact that in the single example of SC deficiency referred to above, secreted IgA levels were very low, whereas the serum IgA level was normal.

The α chain is structured similarly to the γ chain, but contains an extra stretch of 18 amino acids at its carboxy terminus. It shares this feature with μ chains, and it is reasonable to assume that this portion of these chains is critical in the polymerization phenomenon common to both of them.

There are two subclasses of IgA, one predominant in serum (IgA1), the other in secretions (IgA2) (Kunkel and Prendergast, 1966; Feinstein and Franklin, 1966; Vaerman and Heremans, 1966). Interestingly, IgA1 is specifically cleaved by a protease of *Neisseria gonorrhoeae,* and presumably other normal and pathogenic flora, while IgA2 is resistant. The reason for this resistance has been elucidated (Plaut et al., 1974). A 13-residue deletion in the hinge region of IgA2 with respect to IgA1 results in the loss of the proteolytic cleavage site and a reduced susceptibility to enzymatic digestion. Most, although not all, IgA2 molecules lack H-L interchain disulfide bonds; the molecule is stabilized instead by L-L and H-H interchain disulfide bonds as well as by noncovalent interactions.

There is still much to be learned about the normal function of IgA. While monomeric IgA appears to be capable of activating the alternate complement pathway, it is unclear whether the secretory IgA can fix complement or not. Its primary role may be to prevent the absorption of soluble antigens by mucosal surfaces or to interfere with the attachment of microorganisms to cells. Whatever the mechanism, it is clear that locally secreted antibodies impart significant resistance to clinical infection, with a variety of exogenous microorganisms representing the first line of defense against infection. While IgG, IgE, and IgM immunoglobulins may also be present, IgA appears to be the major mediator of local resistance. This form of immune resistance may explain the greater efficacy of oral or aerosol immunization with certain of the infectious agents. A role for secretory IgA in

protecting against absorption of partially digested antigens in the gastrointestinal and respiratory tracts has also been suggested as perhaps desirable in minimizing the risk of food allergy or "autoimmunity" to microbial or other products. In this connection it is of interest that IgA deficiency appears to be associated with an increased risk of connective tissue diseases such as SLE and rheumatoid arthritis, as well as increased serum antibody responses to beef antigens in food.

IgM

IgM is the largest of the polymeric immunoglobulins, usually being a pentamer of the H2L2 structure with one J chain. The five monomeric units are arranged in a radial fashion, linked by disulfide bonds. IgM is, with rare exceptions, found in serum only in the pentameric form, and it is this form that is extremely efficient at agglutinating particular antigens and the fixation of the first component of complement in the classic pathway after binding to antigen. By analogy with IgG, IgM antibodies might be expected to be decavalent, but as a rule five of the sites appear to be masked or of low affinity, giving the molecule an effective valence of only five.

The μ chain is somewhat larger than the α or γ chain, possessing an additional domain (110 residues) and the extra 19 residue carboxy terminal segment it shares with the α chain.

IgM is typically the immunoglobulin produced in the primary response to antigenic challenge. Since a continuing source of antigen is apparently needed to maintain the response, its expression is usually transient, although IgM production can be reinitiated at the time of a secondary response. Because of the rapidity with which IgM antibodies form, their predominant localization within the vascular system, their high efficiency in fixing complement, and the availability of five binding sites, giving them a high functional avidity for antigen, IgM is thought to be the first line of resistance against intravascular infections. Indeed primarily intravascular infections, such as malaria and trypanosomiasis, produce a particularly marked IgM response. A major role has also been ascribed to IgM as a cell-surface receptor of B lymphocytes, wherein it exists as a monomer. IgM is also the main immunoglobulin produced by the fetus, and while the amounts formed are usually small when there is fetal or amniotic fluid infection, substantial IgM responses may occur. Significant amounts of IgM are frequently demonstrable in exocrine secretions; the levels are particularly high if there is secretory IgA deficiency. Interestingly, when there is IgA deficiency the majority of the secreted IgM has associated secretory component (SC), suggesting a mechanism for secretion similar to that for IgA. While IgG and IgE also appear in secretions, they do not contain SC. Monomeric IgM molecules are occasionally demonstrated in serum, particularly in association with autoimmune diseases such as systemic lupus erythematosus, but the significance of this observation is currently unclear.

IgD

IgD is present in human serum in minute concentrations (0.03 mg/ml). Its role is thought to be acted out upon the surface of B cells, accounting for its diminished serum presence. Understandably, structural work has been carried out using human myeloma proteins.

IgD is extraordinarily sensitive to proteolysis, and this sensitivity has hampered structural studies. The δ chain is of somewhat higher molecular weight than the γ chain, but this increase is attributable to carbohydrate (Spiegelberg et al., 1972). IgD has been demonstrated to be a receptor on B lymphocytes, particularly in the bone marrow, peripheral blood, and tonsils, and is presumably intimately (but as yet unclearly) involved in triggering the production of antibodies by these cells (Vitetta et al., 1976). Since IgD immunoglobulins are prominent on neonatal B lymphocytes in apparent association with acquisition of immunocompetency, their role may be that of cell-bound antigen receptors involved in the triggering of antibody synthesis. IgD and IgM immunoglobulins are frequently present on the same B lymphocytes, raising the possibility of collaborative or antagonistic interactions. Serum IgD immunoglobulin has not been shown to fix complement, and it has been very difficult to demonstrate antigen-binding activity. Nonetheless, serum IgD levels have recently been found to be under genetic control, so their possible role in immune responsiveness deserves further exploration. The other possibility is that serum IgD is derived from B lymphocyte membrane-bound IgD and is therefore a re-