Immunoglobulins

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An immunoglobulin is a protein which is composed of a characteristic unit of four polypeptide chains: a pair of identical 'light' chains with a molecular weight of 22,000, consisting of ~214 amino-acid residues which do not contribute at all to class specificity but are common to all the immunoglobulins, and a pair of identical 'heavy' chains, molecular weight 50,000–70,000. The heavy polypeptide chains of the immunoglobulin classes and subclasses vary in amino-acid composition and sequence and it is these variations that give each class or subclass its specificity. These chains are linked by disulphide bridges. Among the classes and subclasses of immunoglobulins, members with antibody activity have been identified. The approximate molecular weights of immunoglobulins range from 160,000 for IgG to 900,000 for the IgM molecule which consists of five monomeric IgM units linked by disulphide bonds to form the IgM molecule. However, monomeric IgM (7S IgM) does exist in the serum of normal individuals as well as in a number of disease states. In man, five distinct classes have so far been identified according to their general properties and the class of their heavy chains; these are IgG, IgA, IgM, IgD, and IgE.

Immunoglobulin Classes

Immunoglobulin G is the major immunoglobulin component of serum and of the body extravascular spaces. Members of this immunoglobulin group neutralize bacterial toxins, enhance the phagocytosis of micro-organisms, and may produce cell lysis by activation of the complement sequence. Analysis of IgG myeloma proteins have revealed the presence of four subclasses, IgG₁, IgG₂, IgG₃, and IgG₄. These subclasses (Table I) vary in amino-acid composition and the number of disulphide bridges present. They also vary in their

TABLE I
IgG SUBCLASSES

	Disulphide Bridges	Complement Fixation	Cross Placenta	Macrophages Binding	Total IgG
IgG ₁	2	+++	++	+++	65–70%
IgG ₂	4	+	–	+	20–23%
IgG ₃	5	+++	++	+++	8%
IgG ₄	2	±	++	±	4%

ability to activate complement, to fix to macrophages, and to cross the placenta.

Immunoglobulin A appears selectively in seromucous secretion tears, nasal secretions, saliva, and gastrointestinal secretions. In these secretions it is present as a dimer with an additional secretory component synthesized by the local secretory epithelium. IgA is also present in the serum but mainly as the basic 7S monomer. The secretory IgA present in colostrum, although not absorbed from the gastrointestinal tract of the newborn, probably helps to control the intestinal flora. Serious neonatal infections are less frequent in babies whose milk intake is from the breast in the first few days of life (Winberg and Wessner, 1971). Although IgA does not appear to activate the classical complement sequence, there is evidence that aggregated IgA can do so via the 'alternate pathway' by acting on C3 proactivator to generate C3a. The two subclasses of IgA are differentiated immunochemically and called IgA₁ and IgA₂. Structurally these subclasses are quite different; IgA₂ lacks the interchain disulphide bonds linking the heavy and light chains which are characteristic of all other immunoglobulins. Because of this, the subclasses can be differentiated by electrophoresis in starch or acrylamide gel by suitable conditions (8M urea, pH3) which dissociates the heavy and light chains of IgA₂. Normally human serum consists of approximately

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90% IgA₁ molecules. About 50% of salivary IgA is IgA₂.

The Immunoglobulins M are also known as macroglobulins because of their common form, consisting of five monomer units attached together to make a structure with a molecular weight of about 900,000. They appear early in immune responses and are probably of much importance in bacterial invasion of the internal environment because of their very efficient agglutinating and cytolytic properties. There are two subclasses of IgM which have been differentiated immunochemically (Franklin and Frangione, 1967). A difference in the peptides has been noted (Franklin and Frangione, 1968).

Immunoglobulin D was first identified because of its occurrence as a myeloma protein. There have been recent reports of antibody activity to antinuclear factor and to bovine serum, albumin being identified in this group, but in general its role is not understood.

Immunoglobulin E has reaginic activity; that is the ability to fix to cells, particularly mast cells, and to provoke the release of histamine, slow-reacting substance, and other agents when it combines with antigen. The intervention of complement is not necessary for this type of reaction. Immunoglobulin E levels are elevated in some forms of asthma, eczema, and parasitic diseases.

Analysis of the amino-acid composition of immunoglobulins from different species, which will

be referred to again below, has revealed similarities. From this information it looks likely that all mammalian species will be shown to possess classes of immunoglobulins similar to those already demonstrated in humans. For example, molecules with properties similar to IgE have been identified in monkeys (Ishizaka, Ishizaka, and Tomio, 1969; Patterson, Roberts, and Pruzansky, 1969), dogs (Patterson *et al*, 1969), and rabbits (Zvaifler and Robinson, 1969).

Although immunoglobulins within a single class or subclass share the same overall structure, they are a heterogeneous mixture of chemically different molecules. Even purified antibodies directed against a simple hapten antigen are a population of diverse molecules (Haber, 1968). This heterogeneity has meant that it was not possible to purify such a complex mixture of proteins in order to determine the amino-acid sequence of antibody to a certain antigen. The sequencing work that has been done, therefore, has used the chemically homogeneous immunoglobulins produced in large quantities by myelomas (plasma cell tumours).

Structure of Immunoglobulins

The basic structural model proposed by Porter in 1962 for IgG has been accepted as the basic unit for all other immunoglobulins. The two identical 'light' polypeptide chains which are part of the basic four-chain unit do not contribute to the class specificity but are common to all the immunoglobulins. In general, there are two types of light chains, kappa or lambda. Most, or all mammalian and avian

FIG. 1. The distribution of kappa and lambda light chains in immunoglobulins of various species.

species (Hood *et al*, 1967), paddlefish (Pollara *et al*, 1968), and some shark species (Goodman *et al*, 1970) have chains homologous to human kappa and lambda light chains. However, there are differences: the ratio of kappa to lambda light chains varies (Fig. 1). In mice approximately 95% of the immunoglobulins contain kappa light chain (McIntire and Rouse, 1970) and the horse appears to have one type of light chain only (Hood *et al*, 1967). The nature of the forces which may have produced different light chain ratios in different species have been discussed by Hood *et al* (1967).

The heavy polypeptide chains of the various immunoglobulin classes differ in amino-acid composition and sequence and it is these differences which give the immunoglobulin its class and subclass specificity in addition to the other phenomena such as ability to fix complement, cross the placenta or fix to homologous skin. The heavy chain for each immunoglobulin is identified by the Greek letter equivalent of the class designation, eg, γ for IgG heavy chain, δ for IgD heavy chain, α for IgA, ϵ for IgE, and μ for IgM.

The Basic Immunoglobulin Structure

A distinctive feature is that half the light chains and three-quarters of the heavy chains are identical in amino-acid composition and position to other light and heavy chains of that type and subtype, apart from the occurrence of genetically polymorphic forms. This area is called the *constant region* (C). The amino-acid half of the light chain and quarter of the heavy chains vary in composition in the *variable region* (V). Some of the features of immunoglobulin structure are described and illustrated in Fig. 2.

The region of the basic immunoglobulin molecule which contains all the interchain disulphide bonds is in the middle of the linear sequence of the heavy chain and has no homologous counterpart in other portions of heavy or light chains. Immunoglobulins are formed by linkage of the heavy and light polypeptide chains through interchain disulphide bonds. The peptide sequence near these bonds may be of importance to the biological properties of the molecule. Widely different interchain patterns occur.

The existence of intrachain disulphide bonds and evidence for the evolution of immunoglobulins by gene duplication prompts the hypothesis (to be discussed later) that each region containing a single intrachain disulphide bond is folded in a compact domain.

From the work of Edelman and his co-workers

(1969) on the amino-acid sequence of IgG1, the following conclusions were made.

(1) The variable parts of the heavy and light chains are homologous to each other, but not to the constant parts. (2) The constant part of the γ 1 chain has three homologous regions C_{H1} , C_{H2} , C_{H3} . These are not homologous to the constant part of the light chain. (3) Each variable region and each constant homology region contains one disulphide bond, each forming a similarly sized loop in the polypeptide chain.

These results provide evidence for the hypothesis that immunoglobulin chains evolved by duplication of a precursor gene which determined a protein of about 110 residues. Because there is no convincing evidence of homology between the V (variable) and C (constant) regions, it is difficult to decide whether or not the V and C regions originally evolved from a single gene. If not, two unrelated genes may have combined together in order to produce a product with greater advantages.

The Domain Hypothesis

The repetition of homologous regions, the periodic arrangement of intrachain disulphide bonds, and the location of the interchain bonds suggest conclusions about the tertiary and quaternary structure of the immunoglobulin molecule. Edelman (1970 and 1971) has proposed that the molecule may be made up of a T-shaped series of compact domains, each formed by a V homology or C homology region. Each domain contains a single intrachain disulphide bond which stabilizes it. The intervening areas between these domains are less tightly folded stretches of polypeptide chain. The function of the V region is to bind antigen and the C_{H2} may play a role in complement fixation. The area between the domains C_{H1} and C_{H2} has no homologous counterpart in the rest of the molecule. It has been demonstrated that this is a 'hinge' region in which the molecule can be bent (Valentine and Green, 1967). It is this area that contains the sites of cleavage.

Three-dimensional Structure of Immunoglobulins

Although some evidence of the nature of the three-dimensional structure of immunoglobulins has come from physico-chemical, chemical, and electron microscopy studies, the details must come eventually from x-ray diffraction analysis of antibody crystals. The basic immunoglobulin molecule appears to consist of three roughly equal parts connected by a flexible hinge. Evidence that under some circumstances the molecule assumes a Y shape has been

FIG. 2. Half an immunoglobulin basic unit. The main features of the heavy and light polypeptide chains (see text).

The Light Chain

A. The amino terminal end of chain: usually glutamic or aspartic acid, but more variable in lambda chains and in these may not have a free alpha amino group.

B. Free carboxyl terminal of kappa peptide chain (212–220 amino-acid residues) is cysteine. In lambda types, serine is the terminal group and cysteine precedes it.

C. This is the end of the variable and start of the constant portion (at approximately residue 108).

D. The disulphide part of the variable region at residue 23 contains 65–69 residues. V_L .

E. The disulphide portion of the constant portion near position 135; it contains 58–60 residues. C_L .

F. In lambda chains the antigenic difference O_z (+) or O_z (–) are correlated with position 190 containing lysine or arginine, respectively. In kappa chains possessing genetic Inv 1,2 activity residue 191 is leucine and valine for Inv 3.

The Heavy Chain

1. The amino terminal end is a glutamic acid in cyclic pyrrolidyl carboxylic acid (PCA) form.

2. The glycine carboxyterminal end represents residue 440.

3. Near the end of a short constant segment of Fd portion of H chain there is the beginning of a more variable segment which continues to amino acid 120. Different sequences for different antibodies observed in this section.

4. Beginning of the more constant region of Fd, but the position is not clearly defined.

5. The first and second cysteine residues of H chain first disulphide loop. The first residue is near residue 21. V_H .

6. Position of the third and fourth cysteine residues. C_H1 .

7. The third disulphide loop of chain and first of Fc fragment which begins at 260 and contains 55–60 residues. C_H2 .

8. The fourth disulphide loop at residue 360 which contains between 55 and 60 residues. C_H3 .

9. The region of cleavage of the chain by papain is at residue 220–225. F_C fragment = 9 to 2 (COOH end); Fab = remainder.

10. The region of initial cleavage by pepsin at residue 245–250.

11. Hinge region where two or more cysteine residues of each H chain separated by proline-proline, are linked by disulphide bonds to the adjacent H chains.

12. Point of attachment of major portion of carbohydrate of IgG. Human IgM has up to five times as much carbohydrate as IgG which are present at multiple sites; at hinge region in Fd and 3 in Fc region.

13. Region showing peptic difference between molecules possession genetic Gm(1) activity.

14. Relatively constant terminal portion of chain; the last 20 residues of horse, rabbit, and human IgG differ only at two portions. The only differences between IgG₁, IgG₂, IgG₃, and IgG₄ are that the second residue from the end is proline in IgG₁, IgG₂, and IgG₃, but is replaced by leucine in IgG₄. The 12th position from the end is histidine in IgG₁ and IgG₂ and is replaced in IgG₃ by arginine.

obtained by electron microscopy studies (Valentine and Green, 1967; Svehag and Bloth, 1970). Porter obtained rabbit Fc fragments as crystals and Poljak *et al* (1967) described x-ray investigations of these and human Fc fragments. More recently a number of human Fab fragments have been crystallized. Terry, Matthews, and Davies (1968) reported that crystals of human γ G1 myeloma protein had cell units of $194\text{\AA} \times 92\text{\AA} \times 52\text{\AA}; \beta = 102^\circ$. However, measurements of low angle x-ray scattering (Pilz *et al*, 1970) have suggested that the molecule in solution has larger dimensions than those measured in the electron microscope.

Sarma *et al* (1971) have suggested four possible models of the immunoglobulin molecule based on four ways of joining the Fab regions to the Fc. Basically, the model is T shaped and the Fab arms can rotate through a restricted arc of about 30° . The Y shaped structure seen by electron microscopy is assumed to be produced by combination with antigen. During these alterations in tertiary structure complement fixing sites (if present) may become exposed in the C_{H_2} domain.

Genetic Systems and Markers

Inherited antigenic markers associated with immunoglobulins have provided a useful tool for the investigation of the genetics of antibody formation. Grubb in 1956 noted that the sera from some patients with rheumatoid arthritis agglutinated red cells coated with certain incomplete Rhesus antibodies but not others. This was due to the Gm antigens or markers. Now, however, antibodies produced by transfusion or pregnancy are used in preference to sera from rheumatoid patients. The presence of Mendelian genetic markers, identified chemically or serologically on several C regions of heavy chains, indicates the existence of a single gene for the corresponding region: $\gamma_1, \gamma_2, \gamma_3$ (Natvig *et al*, 1967; Steinberg, 1969) γ_4 (Vyas and Fudenberg, 1969), α_2 (Kunkel *et al*, 1969; Vyas and Fudenberg, 1969).

Light Chains

Variable Region. A study of amino-acid sequences reveals that although there is one kappa chain C type, the V region sequence can be divided into at least three main subgroups (Milstein, 1967), each of which probably originates from a separate gene or group. Present sequence data increases the number and when more data is available, this number may well rise to 20 or more. Similarly there is one lambda C region type but there are probably four or five subgroups of lambda V region sequences (Hood and Ein, 1968; Hood and Talmage, 1970).

Each light chain, therefore, is the product of two separate genes: one for the kappa or lambda C region and one from a number of kappa or lambda V region genes. Only kappa C region can combine with kappa V region. Lambda C region must combine with lambda V region.

Constant Region. The human kappa chain marker Inv (1,a) or (1,2) and Inv (b) or (3) are associated with a single amino-acid interchange (Baglioni *et al*, 1966; Milstein, 1966). Fingerprint and sequence analysis has shown that Inv antigens are in a constant position in the kappa chain: Inv 1 is associated with a leucine residue in position 191, and Inv 3 is associated with valine. Members of any of the kappa V region subgroups may combine with either Inv 1,2 or Inv 3 kappa C chain, but not with lambda constant region.

An amino-acid interchange in the lambda chain is responsible for the Oz antigenic determinant. At position 190, there is lysine in Oz (+) and arginine in Oz (-) λ chains. However, Oz (+) and Oz (-) chains are both present in normal human serums but monoclonal λ light chains are solely Oz (-) or Oz (+). The Oz (-) form is the commoner, occurring in 75% of monoclonal light chains. The Oz determinant, therefore, probably represents a subgroup of λ rather than a genetic type.

Light Chains			
Variable Region	Constant Region		
— V_{K_I}	— κ	}	Kappa type
— $V_{K_{II}}$			
— $V_{K_{III}}$			
— V_{λ_I}	— λ_{Oz+}	}	Lambda type
— $V_{\lambda_{II}}$			
— $V_{\lambda_{III}}$			
— $V_{\lambda_{IV}}$			
— V_{λ_V}	— λ_{Oz-}		

Heavy Chains			
Variable Region	Constant Region		Immunoglobulin Class
— V_{H_I}	γ_1	}	G
— $V_{H_{II}}$	γ_2		
— $V_{H_{III}}$	γ_3		
	γ_4	}	A
— $V_{H_{IV}}$	α_1		
	α_2	}	M
	μ_1		
	μ_2	}	D
	δ		
	ϵ		E

Fig. 3. Possible combinations of light and heavy chain variable and constant regions.

Heavy Chains. The synthesis of heavy chains differs from that of light chains. Whereas in light chain synthesis, kappa variable (V) region subgroups combine only with kappa constant (C) region and lambda V combines only with a lambda C region, all heavy chain V regions appear to belong to a single heavy chain group and these combine with any heavy chain C region.

Variable Region. Three subgroups have been well defined (Pink and Milstein, 1969; Press and Hogg, 1970) and there is evidence for a fourth (Bennett, 1968). All heavy chain V regions belong to a single group which may associate with any heavy chain constant region. This is shown by the following observations.

(1) The amino-acid sequence of human IgA, IgM, and the subclasses of IgG cannot be distinguished on a basis of the variable region of the heavy chain. They share various V region subclasses that are recognizable by the amino-acid sequence.

(2) Nisonoff *et al* (1971) found that the myeloma protein produced by a patient (Til) was of two types, IgG₂κ and IgMκ. Til IgG and Til IgM shared individual antigenic specificities not attributable to light chains. Their heavy chains belonged to the same subclass of variable region and they had an identical amino-acid sequence up to position 35.

(3) Rabbit immunoglobulins A, G, and M share a heavy chain allotypic marker present in the variable region.

(4) Normal immunoglobulin G and M molecules may be synthesized by members of the same cell line. For example (a) rabbit anti-Salmonella antibodies of IgG and IgM share idiotypic determinants (Oudin and Michel, 1969) and (b) Nossal's group (1964) and Pernis' group (1971) have demonstrated the synthesis of IgG and IgM by the same cell.

(5) The finding of (a) identical or similar genetic markers associated with the variable region of rabbit γ, μ, α, and ε heavy chains (Todd, 1963; Kindt and Todd, 1969) and (b) the direct comparison of the amino-acid sequences of rabbit γ + α chains (Wilkinson, 1969); and that of human γ chains (Press and Hogg, 1970), μ chains (Wikler *et al*, 1969), α chains (Wang *et al*, 1970), and ε chains (Terry, Ogawa, and Kochwa, 1970).

In the heterozygous rabbit there occurs little or no somatic interchange of maternal heavy chain C region and paternal heavy chain V region. It has been suggested that only linked C region genes may share members of V region gene group. Nossal and his co-workers (1964) proposed that an individual cell had the ability to switch from the synthesis of

IgM to IgG antibodies of the same specificity. Recently studies on the light chain and heavy chain sequences of the Myeloma proteins Til IgM κ and Til IgG₂ κ from the patient Til revealed there were identical sequences of amino acids in the light chain and N terminal part of the heavy chain (Wang *et al*, 1970; Pink, Wang, and Fudenberg, 1971).

In general, one cell makes immunoglobulin of one allotype (Pernis *et al*, 1965; Cebra, Colberg, and Dray, 1966), one C region class (subclass) and type (Bernier and Cebra, 1965; Pernis, 1967), and of one antibody specificity only (Nossal and Makela, 1962).

Constant Region. For IgG constant regions of γ₁, γ₂, γ₃, and γ₄ heavy chains are under the control of four different genes. There is a very low incidence of recombination among these genes which suggests that they are closely linked. The studies of Kunkel *et al* (1969) on the crossover of Gm factors suggest that the order is γ₄-γ₂-γ₃-γ₁. IgA and IgM have two genes each, and IgD and IgE probably have one gene.

The Fusion of Constant and Variable Region Genes. The variable and constant part of heavy and light chains may fuse because of combination of (1) the DNA responsible, (2) the messenger RNA, and (3) the chain segments themselves.

In heavy chain disease one form of myeloma, sections which include both the variable and constant part of the heavy chain are missing. This strongly suggests that the defect, and therefore the fusion, is at the level of DNA.

Inv Factors are associated with kappa light chains. Therefore, Inv activity can be found in association with IgG, IgA, IgM, IgD, and IgE molecules.

Studies have shown that the three antigens determined by the Inv locus, Inv (1), Inv (2), and Inv (3) are inherited via three alleles Inv¹, Inv^{1,2}, and Inv³. Each antigen can be detected in the heterozygote. The Inv¹ allele is rare, and nearly all light chains positive for Inv (1) are positive for Inv (2). Though Inv antigens are present on the kappa-type light chain, the antigenicity would appear to depend upon the complex structure of the complete immunoglobulin molecule for its full expression because much higher molar concentrations of isolated light chains than of intact or reconstituted IgG are required to detect Inv (1) or Inv (3) activity. A single amino-acid difference differentiates the Inv (1) and Inv (3). Family studies suggest that the Inv locus and Gm loci are at least 30 crossover units apart and may be on separate chromosomes.

Anti-Inv (1) has been used for nearly all the

population studies so far because of the scarcity of anti-Inv (2) and (3). Inv (1) is most frequent in Melanesians (97%), Venezuelan Indians (94%), and is least frequent in Caucasians (approximately 20%) (Steinberg, 1969). Ninety-eight per cent of Inv (1) samples from Caucasian donors are also Inv (2) and 95% are Inv¹/Inv³ heterozygotes. The observation that a kappa Bence Jones protein or kappa chain from a myeloma protein is Inv (1) or Inv (3) but not both therefore points to the activity of one allele only in a single cell or clone.

Gm Factors are associated with IgG molecules alone, and all the factors so far identified have been located on the Constant regions of γ chains. These antigens are transmitted in groups via co-dominant alleles in a similar manner to the Rhesus antigens. Since the WHO Group Report in 1965, Gm groups have been assigned numbers in the place of the letters used at first (Gma, Gmx, etc).

The Gm alleles found in ethnic groups are markedly different, for example, as follows:

- Caucasian.* Gm^{1, 17, 21}; Gm^{1, 2, 17, 21};
Gm^{3, 5, 13, 14};
Negroid. Gm^{1, 5, 13, 14, 17}; Gm^{1, 5, 14, 17};
Gm^{1, 5, 6, 17}; and Gm^{1, 5, 6, 14, 17}.
Pygmy. Gm^{1, 5, 6, 17}; Gm^{1, 5, 13, 14, 17}.

Different Gm phenotypes have been found in different ethnic groups, therefore, these Gm antigens have been used to study racial migration and admixture. For example, it has been possible to determine the amount of Bushman admixture in Negro tribes in South and South West Africa (Steinberg, 1969) and show that Bushmen originated in the South-East part of Africa. With Gm typing it has been found that American Negroes in Baltimore and Cleveland (Steinberg, Boyer, and Stauffer, 1960) have 30% white admixture. The frequency of Gm(1) is greatest in north-east part of Europe (Finland and North Sweden) and least in South West Europe.

Recent work (Steinberg, 1969) has shown that most of the Gm antigens (1, 2, 5, 6, 7, 8, 9, 10, 11, 14, 15, 16, 18, 19, 20, 21, 22, 23, 24) are present on the Fc fragments of heavy chain IgG. However, Gm (3) and (17) are on the Fab fragment of IgG in the Fd portion of the heavy chain. Among the subtypes of IgG certain Gm antigens are prevalent:

- γ G1. Caucasians: Gm (1) or Gm (1, 2) or Gm (3).
Negroes: Gm (1), Mongoloids Gm (1).
 γ G3. Caucasians: Gm (5, 13, 14) or Gm (21).
Negroes: Gm (5, 3, 14) or Gm (5, 6) or Gm (5, 6, 14).
 γ G2. 8, 9, 18a, 23.

Examination of the amino-acid sequence of Gm (1) and Gm (-1) peptides has been made by Frangione *et al* (1966), Thorpe and Deutsch (1966) and Waxdal, Konigsberg, and Edelman (1967), and the change in amino-acid residues occurs at 87-91 from the C terminal end.

Am System. One factor has been reported for this system. Am (1) is associated with the constant regions of IgA₂ heavy chains.

Biosynthesis and Secretion of Immunoglobulins

For many years the synthesis of immunoglobulins had been attributed to plasma cells and to a lesser degree to lymphocytes. The demonstration by immunofluorescent techniques of immunoglobulin present within plasma cells and to a lesser extent lymphocytes lent support to this. When cells were examined from patients with a normal immunologic system, it was found that one heavy chain and one light chain type could be identified per cell, apart from a small percentage of cells which, using immunofluorescent techniques, appeared to contain more than one heavy chain type (Bernier and Cebra, 1964; Pernis and Chiappino, 1964).

Immunofluorescent studies of immunoglobulin production have produced some very interesting information. The predominance of IgA production over IgM and IgG (ratio 18:2:1) by plasma cells associated with the gut was observed by this method (Crabbé, Carbonara, and Heremans, 1966). Also the synthesis of the additional polypeptide (secretory component) which links two IgA monomeric units to make secretory IgA was shown by these methods to occur not in plasma cells or lymphocytes, but in gut epithelial cells.

Immunoglobulin on the Cell Surface. The presence of immunoglobulin has been demonstrated on the surface of between one third and one quarter normal human lymphocytes by means of immunofluorescent reagents. The predominant heavy chain type is γ and the κ to λ ratio is 2:1. The presence of immunoglobulin on the membrane surface does not appear to be dependent upon the phase of the cell cycle (Lerner and Hodge, 1971) whereas the presence of cytoplasmic immunoglobulin appears to be related to the cell cycle: the bulk of cytoplasmic synthesis of Ig occurs in late G₁ and the S phases of the cell cycle (Buell and Fahey, 1969; Lerner and Hodge, 1971).

Data from hyperimmune rabbit lymph node cells and mouse myeloma cells show that heavy and light

chains are synthesized on separate polyribosomes of 300S and 200S, respectively (Askonas and Williamson, 1966; Shapiro *et al*, 1966). Initially, the combination of the L and H chains may take place while the H chain is still on the polyribosome but most probably occurs in the cisternae of the endoplasmic reticulum. Although work on rodents suggests that antibody is mainly synthesized on membrane bound ribosomes (Vassalli, Lisowska-Berstein, and Lamm, 1971), there is some evidence derived from human cells that heavy chains may be synthesized on free cytoplasmic particles (Sherr and Uhr, 1970). The morphology of immunoglobulin producing cells as revealed by the electron microscope varies and while plasma cells have a rough endoplasmic reticulum, lymphocytoid cells may have little or none. It would appear likely, therefore, that immunoglobulin can be produced by either membrane-bound, or free, polysomes.

The synthesis of antibody molecules is similar to that of other proteins. It has been suggested that the polypeptide chains are synthesized as a separate V+C region, but some workers believe that light and heavy chains are synthesized from a single starting point. Heavy and light chains are synthesized on two different size classes of polyribosomes, both in rabbit lymph nodes and in mouse plasma cell tumours. The polysomes coded for heavy chains contain between 16–20 ribosomes. A haemoglobin chain with a molecular weight of 16,000 is made on a polysome of 5 ribosomes. The sizes of the ribosome complexes for immunoglobulin synthesis suggest that each messenger RNA is sufficiently long to code for an entire length of light or heavy chain.

There is probably balanced synthesis of heavy and light chain in normal immunoglobulin producing cells and free light chains are probably intermediate in the assembly of the whole molecule and may attach to heavy chains which are still attached to the ribosomes. There is much data available on the synthesis and assembly of human, rabbit, and mouse IgG and several methods are used in the production of the basic H₂ L₂ unit. H and L chains may combine by disulphide linkage with subsequent dimerization of the HL sub-unit. Another way is by formation of H₂ first with subsequent light-heavy chain disulphide formation. The method of assembly for members of a subclass appears similar, and therefore may reflect the force of interaction between Constant regions of the chains. Initially, on the basis of studies on mouse tumours and rabbit lymph node cells, it was thought that excess light chain production occurred in IgG synthesis. Studies on IgG myeloma in some human

patients have demonstrated balanced synthesis (Sutherland, Zimmerman, and Kern, 1972). However, of IgG myelomas, 75% studied have produced excess light chains but because of the ability of the body to rapidly catabolize light chains, not all of these myeloma patients show excess light chains in the urine.

The IgM molecule (19S) is of large size and there was some doubt as to whether or not it is produced intracellularly. However, in recent studies on cells obtained from patients with macroglobulinaemia, three quarters were found to contain the pentameric form of IgM intracellularly (Buxbaum *et al*, 1971).

The assembled molecules of immunoglobulin are confined to the microsomal part of the lymph node cells but immunoglobulin with carbohydrate attached is found in cell gap. The complete carbohydrate chain is slowly synthesized over the 15–20 minute period between completion of synthesis of protein portion and appearance of the completed immunoglobulin molecule in the extra cellular medium. Whether or not carbohydrate incorporation is obligatory to the normal secretory process is not yet determined.

The amount of DNA in mammalian lymphoid cell is so large (Gurvichi and Nezlin, 1965) that 1% of the DNA could contain 30,000 different genes for the same number of heavy chains, and also the same number of genes for light chain: thus, the amino-acid sequences of approximately $30,000 \times 30,000$ (10^9) antibody molecules could be determined. Experiments directed at determining the assembly time of immunoglobulin molecules in a lymphoid cell give an estimate of approximately 120/min (240 H chains). The time for H-chain formation is 1 min, and combination takes place spontaneously within a few seconds. Since the cell produces approximately 240 molecules of H chain/min, there must be at least 240 H-chain forming sites, whose nucleotide sequences are determined by the transcription of the same numbers of cistrons (genes). Only a very small amount of the total DNA is translated into mRNA and involved in antibody formation. If 1% of total DNA contains 30,000 cistrons for H chains, then the 240 cistrons actually involved = 0.008% of total DNA of the cell. The number involved may be even smaller because one cistron may produce more than one molecule of mRNA per minute. The secretion of free light chains by actively secreting plasma cells takes about 20 minutes from the time of synthesis. Fully assembled IgG does not appear until 30–40 minutes after synthesis (Laskov, Lanzerotti, and Scharff, 1971).

Immunoglobulin Levels

Wide ranges of plasma immunoglobulin levels are found in healthy adults and children. These variations are produced by both environmental and genetic factors, but evidence from germ-free animals in which the immunoglobulin levels are much lower than in normal animals points to environmental factors in animals being the predominant influence. Some of the factors influencing plasma levels are discussed below.

Genetic. Studies on monozygotic and dizygotic human twins (Rowe, Boyle, and Buchanan, 1968a) point to the existence of genetic control, but suggest that the effect of this is relatively small. Male monozygotic twins had a significantly smaller difference in IgG levels than dizygotic twins. The notable feature of this study was that evidence for genetic variation was confined to IgG in males. In adolescents evidence existed for some regulation of IgA and IgM levels, but any effect was small enough to be detectable in the adult population.

However, striking examples of familial incidence of selective immunoglobulin deficiency arouse interest in the genetic aspects. For example, the selective deficiency of immunoglobulin A occurs in approximately one in 500 persons. The association of IgA deficiency and certain 'autoimmune' diseases, eg, rheumatoid arthritis and lupus erythematosus, is much more frequent than the chance occurrence which is estimated to be less than 1 in 10,000 (Hong and Ammann, 1972). These observations have led to the systematic study of patients with IgA deficiency for autoimmune phenomena, and examples of autoimmune phenomena segregating with IgA deficiency in family studies have been reported. Autosomal dominant, autosomal recessive, and polygenic phenomena have been postulated to account for familial cases of IgA deficiency. The latter explanation now seems more likely in view of the frequent occurrences of sporadic cases; the complete absence of IgA, low IgA, and normal IgA levels in members of the same family; and the lack of uniform response (Grundbacher, 1972). However, the isolated absence of IgA occurs in a number of normal people, ie, with no demonstrable ill effects from this phenomena. It has been estimated that the selective absence of an immunoglobulin class occurs in 1 in 200 random hospital admissions.

In man, race also appears to be an influence on IgG (Turner and Voller, 1966; Lichtman, Vaughan, and Hames, 1967; Rowe *et al*, 1968b) and IgM levels (Turner and Voller, 1966). For example, Negroes have a higher IgG and IgM than Caucasians. Wells (1968) showed that in one area of New

Guinea Watut aborigines have a higher level of IgG and IgM than non-Watut aborigines and that both have higher levels than Caucasians.

Sex also appears to be a factor in influencing levels. In some populations (Lichtman *et al*, 1967; Rowe *et al*, 1968b) the IgM level is greater in females than males, but not in other populations (Rowe *et al*, 1968b). In a Dutch population, Stoop *et al* (1969) also found that IgG was susceptible to sex factors; and, as an interesting sidelight on this, it has been observed that boys are more susceptible to infection than girls (Washburn, Medearis, and Childs, 1965). In a study of normal women (46,XX), normal men (46,XY) and patients with dysgenetic ovaries (45,X), Wood and colleagues (1969) found that the IgM concentration was higher in those with XX and the levels in 45,X and XY were similar. The suggestion that the level of IgM is influenced by the number of X chromosomes is supported by the high levels recorded in 46,XXX subjects (Rhodes *et al*, 1969).

Age. There is a rise in IgG and IgA levels with increasing age (Stoop *et al*, 1969). No increase was detectable by Stoop *et al* (1969) for IgM during childhood but the level was below that of adults. The IgA level even at 12 years old was still not near the adult level. A fall of immunoglobulin levels at 12 years has been observed by Stiehm and Fudenberg (1966) and Stoop *et al* (1969) and might be connected with the onset of puberty. Its cause is not known. In healthy adults the levels of three major immunoglobulins remain stable for long periods of time (West, Hong, and Holland, 1962; Allansmith, McClellan, and Butterworth, 1967).

Climate. It has been suggested that low IgG and IgM levels found in Mexico City residents (at 2240 m above sea level) compared with the levels of those who dwell by the coast (at Acapulco) are due to the effect of altitude (Alarcon-Segovia and Fishbein, 1970). McGregor *et al* (1970) measured the IgG, IgA, IgM, and IgD concentrations of a rural Gambian community on three occasions over a 13-month period. Malarial parasitaemia was found to be associated with an elevated IgG level in the age groups up to 20 years, but no constant relationship was found with IgA and IgD. The IgM level was increased when parasitaemia was present only in the first two years of life.

Pregnancy. The Gambian survey (McGregor *et al*, 1970) included 133 pregnant women; the mean IgG and IgA values were significantly lower ($p=0.001$) than those for non-pregnant women.

IgG appeared to fall progressively throughout pregnancy and reached its lowest value during the last 10-week period. This study, and that of Allansmith *et al* (1967), revealed that immunoglobulin levels within individuals appear to be relatively stable.

This review has attempted to deal with some aspects of immunoglobulin structure and synthesis, genetic markers, and some of the factors that influence the immunoglobulin concentration in human blood. For further information the reader is referred to reviews by Steinberg (1969), Edelman (1971) and Pink *et al* (1971).

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