

# IMMUNOLOGY

THIRD EDITION

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## Antigen Receptor Molecules

The recognition of foreign antigen is the hallmark of the specific adaptive immune response. Two distinct types of molecules are involved in this process – the immunoglobulins and the T-cell antigen receptors (TCR) (Fig. 4.1). Diversity and heterogeneity are characteristic features of these molecules. In both cases there is evidence of extensive gene rearrangements which generate different immunoglobulins or TCRs, able to recognize many different antigens.

The immunoglobulins are a group of glycoproteins present in the serum and tissue fluids of all mammals. Some are carried on cell surfaces where they act as receptors. Others (antibodies) are free in the blood or lymph. They are produced in large quantities by plasma cells, which develop from precursor B cells. Plasma cell is the original histological term used to describe antibody-forming cells (AFCs) seen in blood and tissues. Precursor B cells carry membrane-bound immunoglobulin of the same binding specificity as that produced by the AFC. Contact between these B cells and foreign antigen is needed for induction of antibody formation.

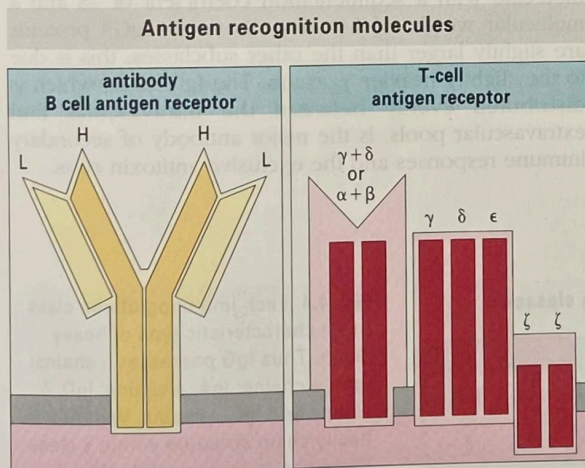


**Fig. 4.1** The antigen receptors of T and B cells are probably derived from a common ancestor and both belong to the immunoglobulin supergene family. Immunoglobulin consists of two identical heavy (H) chains and two identical light (L) chains. The T-cell receptor has an antigen-binding portion consisting of an  $\alpha$  and  $\beta$  chain (or a  $\gamma$  and  $\delta$  chain) which are associated with four other transmembrane peptides ( $\gamma$ ,  $\delta$ ,  $\epsilon$  and  $\zeta$ ), structurally distinct from the chains of the receptor. Circulating antibodies are structurally identical to B cell antigen receptors except that they lack the transmembrane and intracytoplasmic sections.

T-cell receptors are expressed exclusively on the membrane surface of T lymphocytes; there is no production of soluble molecules, analogous to the circulating antibodies. T-cell recognition of antigen through the T-cell receptor is the basis of a range of immunological phenomena including T-cell helper and suppressor activity, cytotoxicity and, possibly, NK (natural killer) activity.

### IMMUNOGLOBULINS

Five distinct classes of immunoglobulin molecule are recognized in most higher mammals, namely IgG, IgA, IgM, IgD, and IgE. These differ in size, charge, amino acid composition and carbohydrate content.

In addition to the difference between classes, the immunoglobulins within each class are also very heterogeneous. Electrophoretically the immunoglobulins show a unique range of heterogeneity which extends from the  $\gamma$  to the  $\alpha$  fractions of normal serum (Fig. 4.2). These fractions suffer marked depletion following

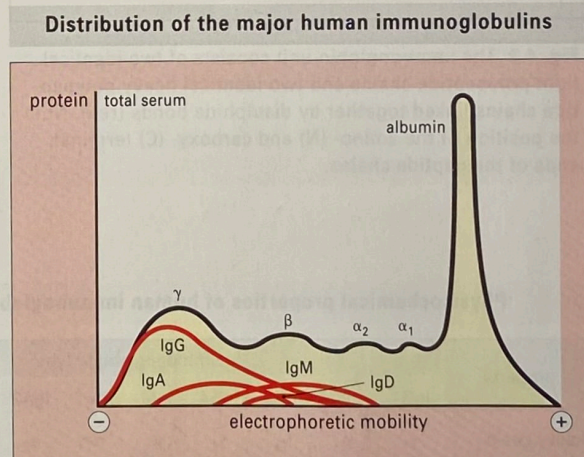


**Fig. 4.2** Electrophoresis of human serum showing the distribution of the four major immunoglobulin classes. Serum proteins are separated according to their charges in an electric field, and classified as  $\alpha_1$ ,  $\alpha_2$ ,  $\beta$ , and  $\gamma$ , depending on their mobility. (The IgE class has a similar mobility to IgD but cannot be represented quantitatively because of its low level in serum.) IgG exhibits most charge heterogeneity, the other classes having a more restricted mobility in the  $\beta$  and fast  $\gamma$  regions.

absorption with antigen. The basic four-chain polypeptide structure of the immunoglobulin molecule is shown in Fig. 4.3.

### ANTIBODY FUNCTION

Each immunoglobulin molecule is bifunctional. One region of the molecule is concerned with binding to antigen while a different region mediates so-called effector functions. These include binding of the immunoglobulin to host tissues, including various cells of the immune system, some phagocytic cells, and the first component (C1q) of the classical complement system.

The basic chain structure of immunoglobulins

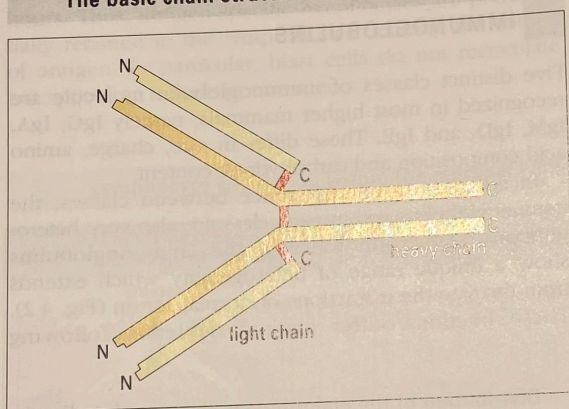


**Fig. 4.3** The immunoglobulin unit consists of two identical light polypeptide chains and two identical heavy polypeptide chains linked together by disulphide bonds (red). Note the position of the amino- (N) and carboxy- (C) terminal ends of the peptide chains.

### IMMUNOGLOBULIN CLASSES AND SUBCLASSES

The basic structure of all immunoglobulin molecules is a unit consisting of two identical light polypeptide chains and two identical heavy polypeptide chains. These are linked together by disulphide bonds. The class and subclass of an immunoglobulin molecule (i.e. its isotype) are determined by its heavy chain type. Thus the four human IgG subclasses (IgG1, IgG2, IgG3 and IgG4) have heavy chains called  $\gamma_1$ ,  $\gamma_2$ ,  $\gamma_3$  and  $\gamma_4$  which differ slightly, although all are recognizably  $\gamma$  heavy chains.

The four subclasses of human IgG (IgG1, IgG2, IgG3 and IgG4) occur in the approximate proportions of 66%, 23%, 7% and 4%, respectively. There are also known to be subclasses of human IgA (IgA1 and IgA2), but none have been described for IgM, IgD, and IgE.

Immunoglobulin subclasses appear to have arisen late in evolution and the human subclasses cannot be compared with, for example, the four known subclasses of IgG which have been identified in the mouse.

### GENERAL PROPERTIES OF IMMUNOGLOBULINS

All immunoglobulins are glycoproteins, but the carbohydrate content ranges from 2–3% for IgG to 12–14% for IgM, IgD, and IgE. The physicochemical properties of the immunoglobulins are summarized in Fig. 4.4.

IgG is the major immunoglobulin in normal human serum, accounting for 70–75% of the total immunoglobulin pool. IgG consists of a single immunoglobulin molecule with a sedimentation coefficient of 7S and a molecular weight of 146 000. However, IgG3 proteins are slightly larger than the other subclasses; this is due to the slightly heavier  $\gamma_3$  chain. The IgG class, which is distributed evenly between the intravascular and extravascular pools, is the major antibody of secondary immune responses and the exclusive antitoxin class.

Physicochemical properties of human immunoglobulin classes

| property                      | immunoglobulin type |            |            |            |       |            |            |                     |          |            |
|-------------------------------|---------------------|------------|------------|------------|-------|------------|------------|---------------------|----------|------------|
|                               | IgG1                | IgG2       | IgG3       | IgG4       | IgM   | IgA1       | IgA2       | slgA                | IgD      | IgE        |
| heavy chain                   | $\gamma_1$          | $\gamma_2$ | $\gamma_3$ | $\gamma_4$ | $\mu$ | $\alpha_1$ | $\alpha_2$ | $\alpha_1/\alpha_2$ | $\delta$ | $\epsilon$ |
| mean serum conc. (mg/ml)      | 9                   | 3          | 1          | 0.5        | 1.5   | 3.0        | 0.5        | 0.05                | 0.03     | 0.000 05   |
| sedimentation constant        | 7s                  | 7s         | 7s         | 7s         | 19s   | 7s         | 7s         | 11s                 | 7s       | 8s         |
| mol. wt ( $\times 10^3$ )     | 146                 | 146        | 170        | 146        | 970   | 160        | 160        | 385                 | 184      | 188        |
| half-life (days)              | 21                  | 20         | 7          | 21         | 10    | 6          | 6          | ?                   | 3        | 2          |
| intravascular distribution(%) | 45                  | 45         | 45         | 45         | 80    | 42         | 42         | trace               | 75       | 50         |
| carbohydrate(%)               | 2–3                 | 2–3        | 2–3        | 2–3        | 12    | 7–11       | 7–11       | 7–11                | 9–14     | 12         |

**Fig. 4.4** Each immunoglobulin class has a characteristic type of heavy chain. Thus IgG possesses  $\gamma$  chains; IgM,  $\mu$  chains; IgA,  $\alpha$  chains; IgD,  $\delta$  chains and IgE,  $\epsilon$  chains. Variation in heavy chain structure within a class gives rise to immunoglobulin subclasses. For example, the human IgG pool consists of four subclasses reflecting four distinct types of heavy chain. The properties of the immunoglobulins vary between the different classes. Note that in secretions, IgA occurs in a dimeric form (slgA) in association with a protein chain termed the secretory piece. Serum concentration of slgA is very low, whereas the level in intestinal secretions can be very high.

Secretory IgA (sIgA), which may be of either subclass (IgA1 or IgA2), exists mainly in the 11S, dimeric form and has a molecular weight of 385 000. Secretory IgA is **IgM** accounts for approximately 10% of the immunoglobulin pool. The molecule has a pentameric structure in which individual heavy chains have a molecular weight of approximately 65 000 and the whole molecule has a molecular weight of 970 000. IgM is largely confined to the intravascular pool and is the predominant 'early' antibody, frequently seen in the immune response to antigenically complex infectious organisms.

**IgA** represents 15–20% of the human serum immunoglobulin pool. In man more than 80% of IgA occurs as a monomer of the four-polypeptide chain unit, but in most mammals the IgA in serum is mainly polymeric, occurring mostly as a dimer. IgA is the predominant immunoglobulin in seromucous secretions such as saliva, colostrum, milk, and tracheobronchial and genitourinary secretions.

The basic structure of IgG1

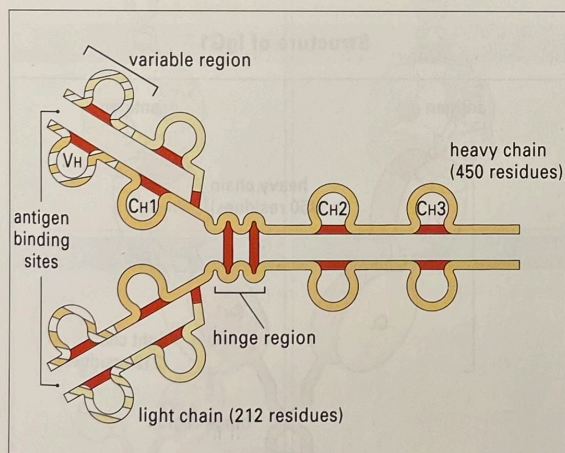


**Fig. 4.5** The N-terminal end of IgG1 is characterized by sequence variability (V) in both the heavy and light chains, referred to as the VH and VL regions respectively. The rest of the molecule has a relatively constant (C) structure. The constant portion of the light chain is termed the CL region. The constant portion of the heavy chain is further divided into three structurally discrete regions: CH1, CH2 and CH3. These globular regions, which are stabilized by intrachain disulphide bonds, are referred to as 'domains'. The sites at which the antibody binds antigen are located in the variable domains. The hinge region is a segment of heavy chain between the CH1 and CH2 domains. Flexibility in this area permits the two antigen-binding sites to operate independently. There is close pairing of the domains except in the CH2 region (see Fig. 4.8). Carbohydrate moieties are attached to the CH2 domains.

abundant in seromucous secretions where it is associated with another protein – the secretory component.

**IgD** accounts for less than 1% of the total plasma immunoglobulin but is present in large quantities on the membrane of many B cells. The precise biological function of this class is unknown but it may play a role in antigen-triggered lymphocyte differentiation.

**IgE**, though scarce in serum, is found on the surface membrane of basophils and mast-cells in all individuals, and also sensitizes cells on mucosal surfaces such as the conjunctival, nasal and bronchial mucosa. This class of immunoglobulin may play a role in immunity to helminthic parasites, but in developed countries is more commonly associated with allergic diseases such as asthma and hay fever.

### ANTIBODY STRUCTURE

In 1962 Rodney Porter proposed a basic four-chain model for immunoglobulin molecules (Fig. 4.5), based on two distinct types of polypeptide chain. The smaller (light) chain has a molecular weight of 25 000 and is common to all classes, whereas the larger (heavy) chain has a molecular weight of 50 000–77 000 and is structurally distinct for each class or subclass. The polypeptide chains are linked together by covalent and non-covalent forces.

In the most abundant immunoglobulin, IgG, the hinge region can undergo proteolytic cleavage by papain to produce two identical fragments that can bind antigen – Fab (fragment antigen binding) and a third fragment which cannot bind antigen – Fc (fragment crystallizable).

Pepsin leaves at a different point and produces an  $F(ab')_2$  and an Fc portion. The  $F(ab')_2$  is divalent and can bind to antigen similarly to the original antibody (see Fig 4.19).

The light chains of most vertebrates have been shown to exist in two distinct forms called kappa ( $\kappa$ -type) and lambda ( $\lambda$ -type). They may be distinguished by their behaviour as antigens – antisera may be raised to one type which do not react with the other. Either of the light chain types may combine with any of the heavy chain types, but in any one molecule both light chains are of the same type – hybrid molecules do not occur naturally.

Hiltschmann, Craig and others in 1965 established that light chains consist of two distinct regions. The C-terminal half of the chain (approximately 107 amino acid residues) is constant except for certain allotypic and isotypic variations (see below) and is called the CL (Constant:Light chain) region, whereas the N-terminal half of the chain shows much sequence variability and is known as the VL (Variable:Light chain) region.

The IgG molecule may be considered as a typical example of the basic antibody structure. IgG has two intrachain disulphide bonds in the light chain – one in the variable region and one in the constant region (Figs 4.6 and 4.7). There are four such bonds in the heavy

( $\gamma$ ) chain, which is twice the length of the light chain. Each disulphide bond encloses a peptide loop of 60–70 amino acid residues, and if the amino acid sequences of these loops are compared a striking degree of homology is revealed. Essentially this means that each immunoglobulin peptide chain is composed of a series of globular regions with very similar secondary and tertiary structure (folding). This is shown for the light chain in Fig. 4.6. The peptide loops enclosed by the disulphide bonds represent the central portion of a 'domain' of about 110 amino acid residues. In both the heavy and the light chains the first of these domains corresponds to the variable region,  $V_H$  and  $V_L$  respectively (Fig. 4.7). In the heavy chain of IgG, IgA and IgD there are three further domains, which make up the constant part of the chain,  $CH1$ ,  $CH2$  and  $CH3$ . In both  $\mu$  and  $\epsilon$  chains there is an additional domain after  $CH1$ . Thus, the C-terminal domains of IgM and IgE heavy chains (referred to as  $C\mu4$  and  $C\epsilon4$ ) are homologous to the  $CH3$  domain of IgG ( $C\gamma3$ ).

Fig. 4.7 shows the structure of the IgG1 molecule in a form which corresponds more closely to the actual molecule than Fig. 4.5. Although Fig. 4.7 is a useful

model for all immunoglobulins, there are differences of detail between every class, and between subclasses.

**IgG** With human IgG, no two subclasses are identical in the number and distribution of interchain disulphide bonds. Indeed, the light-heavy chain bonds in IgG2, IgG3 and IgG4 are located at the junction between the variable and constant regions of the heavy chains and in this pattern is the one most frequently observed in other classes. Similarly, the number of inter-heavy chain bonds may be two (IgG1 and IgG4), four (IgG2) or five (IgG3) (Fig. 4.8).

**IgM** Although usually a pentamer (Fig. 4.9), there is evidence that in both mouse and man some IgM is hexameric. The  $\mu$  chains differ from the  $\gamma$  chains in amino acid sequence and have an extra constant region domain. The subunits are linked by disulphide bonds between the  $C\mu3$  domains and possibly by disulphide bonds between the C-terminal 18-residue peptide tail-pieces. The complete molecule consists of a densely packed central region with radiating arms, as seen in electron micrographs. Photographs of IgM antibodies

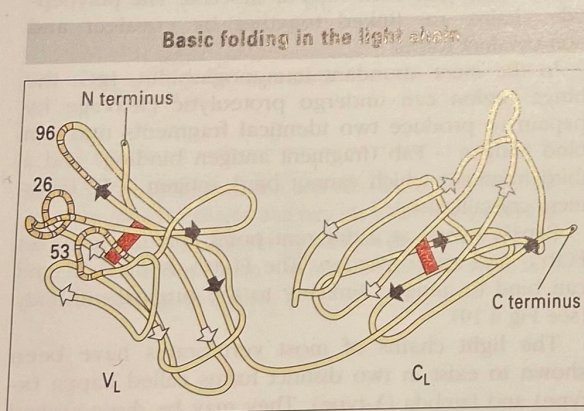


**Fig. 4.6** The basic folding pattern of the variable and constant domains of the light chain. Within the past decade the tertiary structure of immunoglobulins has been investigated by X-ray diffraction techniques. Such studies have shown that the immunoglobulin domains share a basic folding pattern with several straight segments of polypeptide chain lying parallel to the long axis of the domain. Shown here is a light chain which has two domains – one constant and one variable. Within each domain, the polypeptide chain is arranged in two layers, running in opposite directions, with many hydrophobic amino acid side-chains between the layers. One of the layers has four segments (arrowed white), the other has three (arrowed black); both are linked by a single disulphide bridge (red). Folding of the  $V_L$  domains causes the hypervariable regions to become exposed in three separate but closely disposed loops. One numbered residue from each hypervariable region is identified.

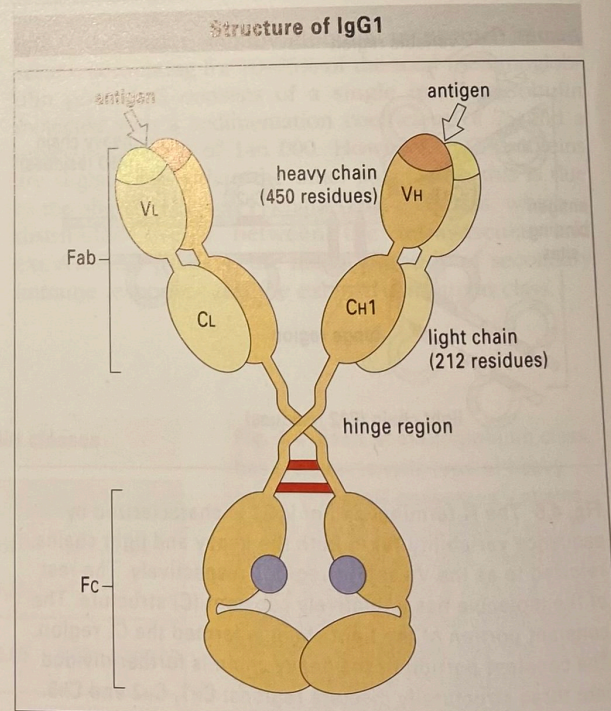


**Fig. 4.7** A model of IgG1 indicating the globular domains of heavy (H) and light (L) chains. Note the apposition of the  $CH3$  domains and the separation of the  $CH2$  domains. The carbohydrate units (purple) lie between the  $CH2$  domains. In this figure (and in Fig. 4.9 onwards) the interchain disulphide bonds between H and L chains are not shown.

binding to bacterial flagella show molecules adopting a 'staple' configuration (Fig. 4.10). The latter suggests that flexion readily occurs between the  $C\mu 2$  and  $C\mu 3$  domains although this region is not structurally homologous to the IgG hinge. The dislocation resulting in the 'staple' configuration appears to be related to the activation of complement by IgM.

Two other features characterize the IgM molecule: an abundance of oligosaccharide units associated with the  $\mu$  chain, and an additional peptide chain, the J (joining) chain, thought to assist the process of polymerization prior to secretion by the AFC. The J chain is

a cysteine-rich peptide of 137 amino acid residues. One J chain is incorporated into the IgM structure by disulphide bonding to the 18-residue peptide tailpiece of the separate monomers. Binding is to the penultimate cysteine residues of the tailpieces. If J chains are not freely available, hexameric IgM appears to become the preferred form, rather than pentameric IgM.

#### Structures of human IgG subclasses

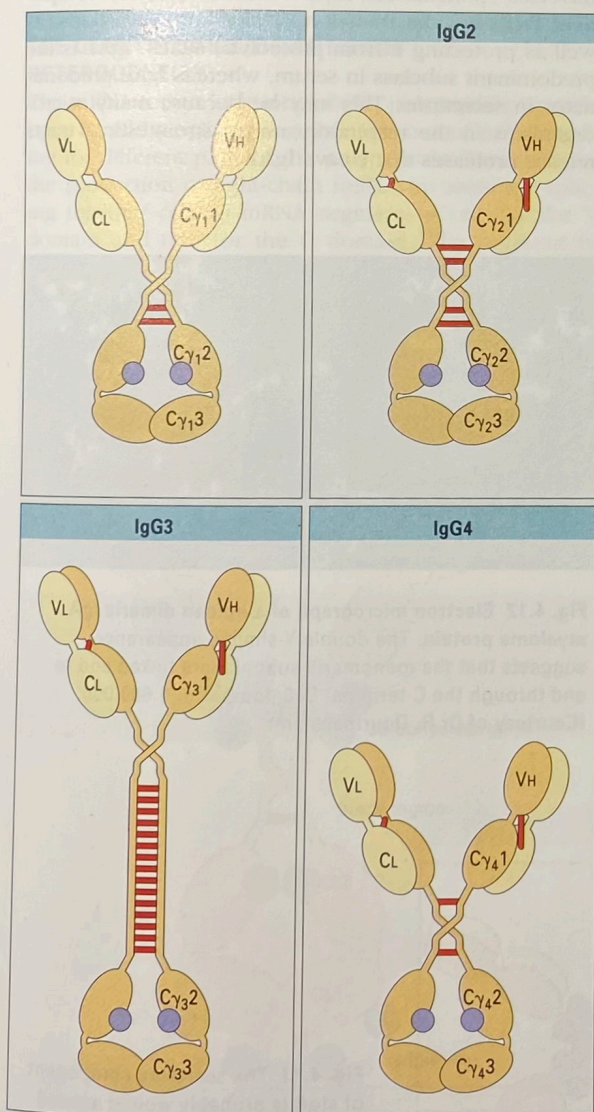


**Fig. 4.8** Polypeptide chain structure of the four human IgG subclasses. The subclasses differ in the number and arrangement of the interchain disulphide bonds.

#### Structure of human IgM

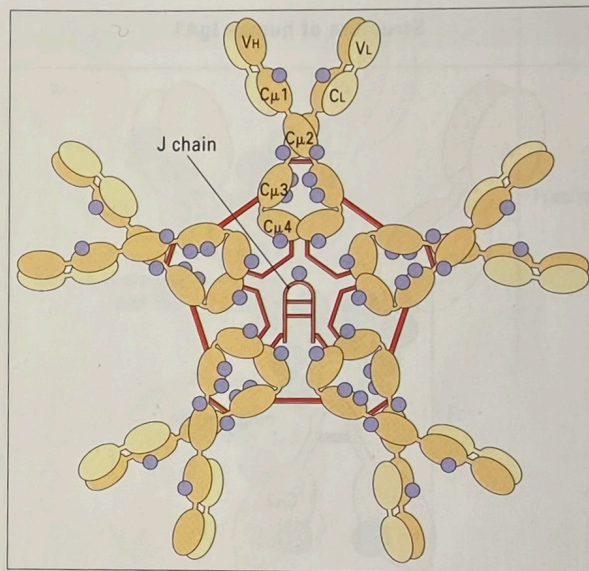


**Fig. 4.9** IgM heavy chains have five domains with disulphide bonds cross-linking adjacent  $C\mu 3$  and  $C\mu 4$  domains. Also shown are the carbohydrate side-chains (purple) and possible location of the J chain. IgM does not have extended hinge regions, but flexion can occur about the  $C\mu 2$  domains.

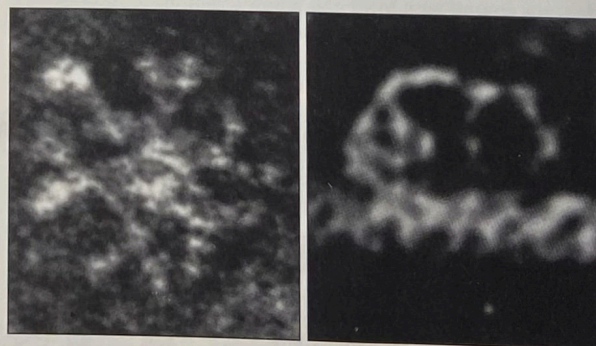


**Fig. 4.10** Electron micrographs of IgM molecules. Left: In free solution, IgM adopts the characteristic star-shaped configuration.  $\times 5\,200\,000$ . (Courtesy of Dr R. Dourmashkin.) Right: IgM bound to a single flagellum has adopted the crab-like, 'staple' configuration.  $\times 5\,200\,000$ . (Courtesy of Dr A. Feinstein.)