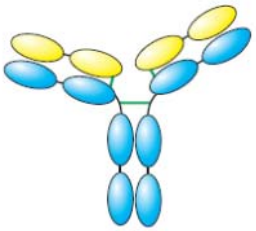
Lesson 2.2 vs 2

The Immune System

Antibodies

Just as Medieval defenders used their weapons and the castle walls to defend their city, the immune system constantly battles against foreign invaders such as viruses, bacteria, and parasites to defend the organism. Antibody molecules provide a key element in the immune system's defensive arsenal. For example, specific antibodies can bind to molecules on the surfaces of viruses and prevent the viruses from infecting cells. Above, an antibody binds to one subunit on hemagglutinin from the surface of influenza virus. [(Left) The Granger Collection.]



Antibodies

The immunoglobulins are a group of glycoproteins present in the serum and tissue fluids of all mammals. Some are carried on the surface of B cells, where they act as receptors for specific antigens. Others (antibodies) are free in the blood or lymph. Contact between B cells and antigen is needed to cause the B cells to develop into antibody forming cells (AFCs), also called plasma cells, which secrete large amounts of antibody. ('Plasma cell' is the original histological term used to describe AFCs seen in blood and tissues.) The membrane-bound immunoglobulin on a precursor B cell has the same binding specificity as the antibody produced by the mature AFC (*Fig. 4.1*).

IMMUNOGLOBULINS – A FAMILY OF PROTEINS

Five distinct classes of immunoglobulin molecule are recognized in most higher mammals, namely IgG, IgA, IgM, IgD and IgE. They 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 γ to the α fractions of normal serum

Distribution of the major human immunoglobulins

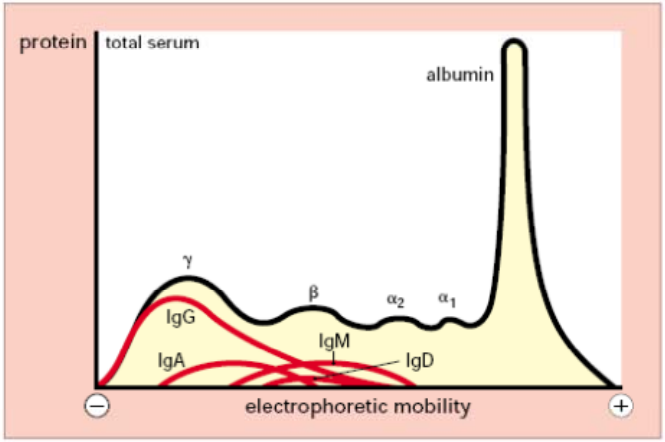
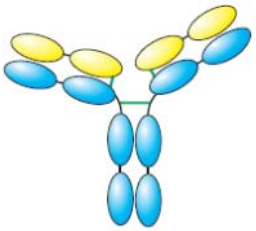


Fig. 4.2 Electrophoresis of human serum showing the distribution of the four major immunoglobulin classes. Serum proteins are separated according to their charges in an electric field, and classified as α_1, α_2, β and γ, depending on their mobility. (The IgE class has a similar mobility to IgD but cannot be represented quantitatively because of its low level in serum.) IgG exhibits the most charge heterogeneity, the other classes having a more restricted mobility in the β and fast γ regions.

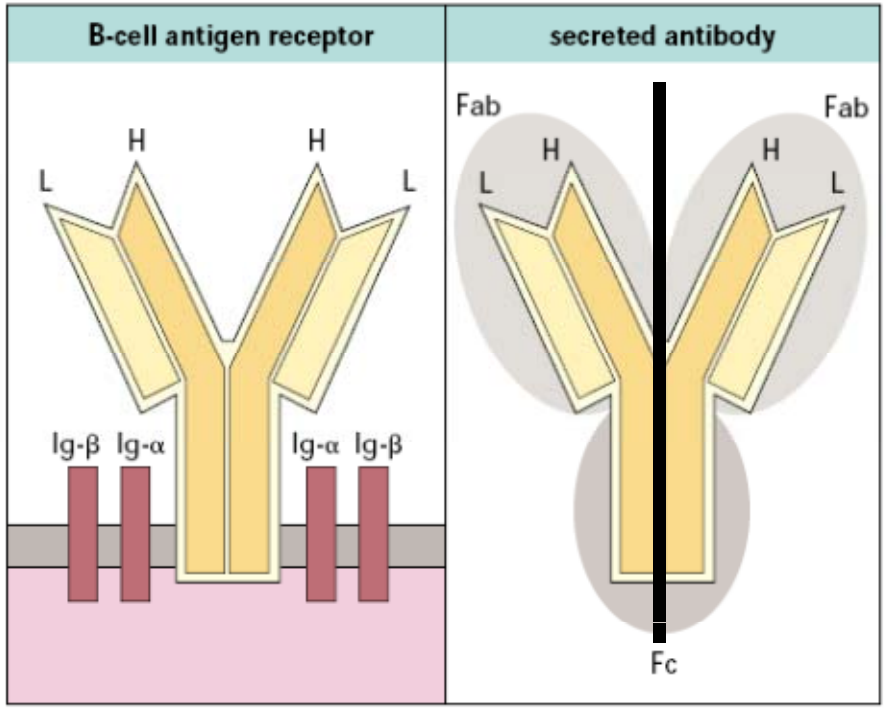


Antibodies

Immunoglobulins are bifunctional molecules

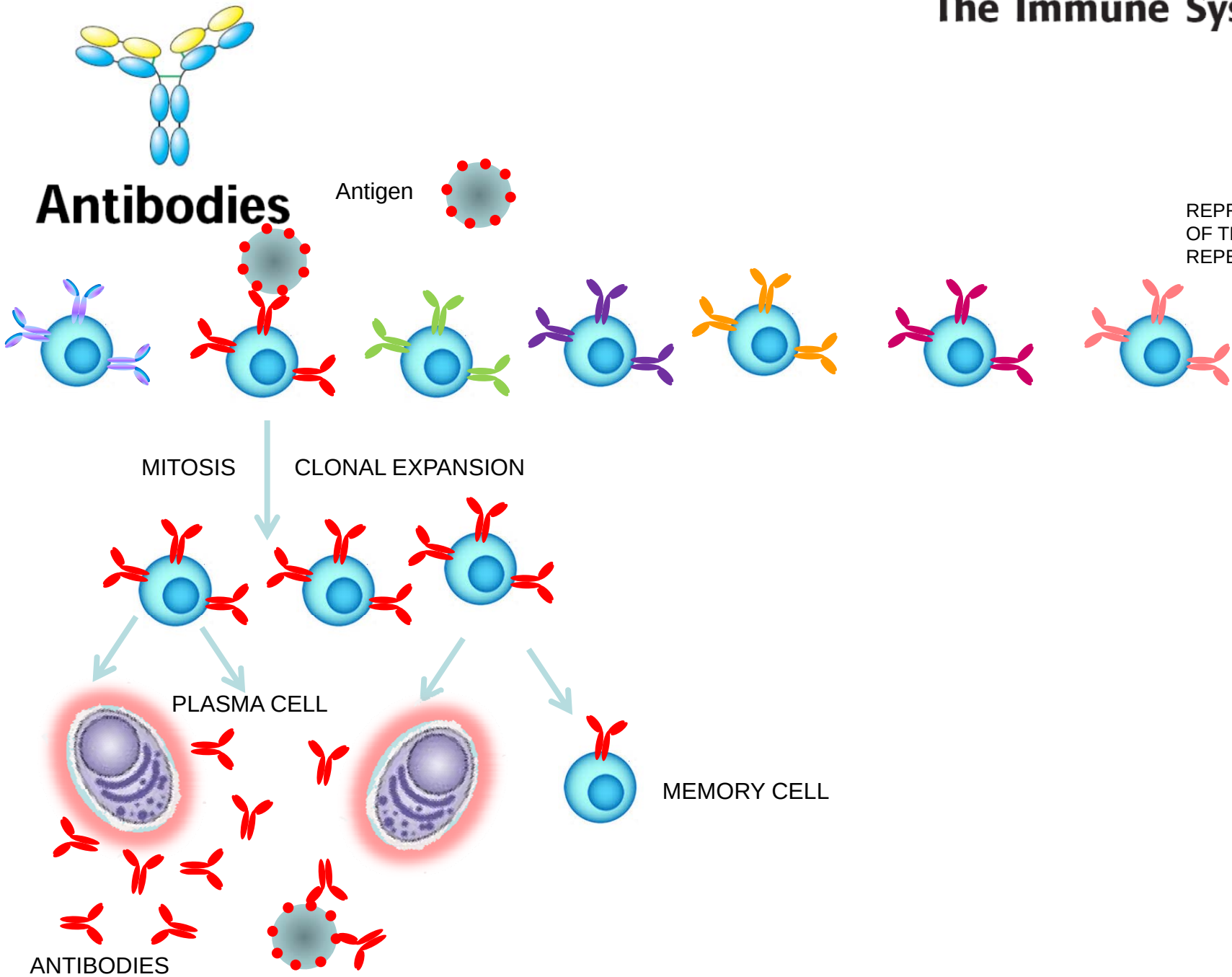
Each immunoglobulin molecule is bifunctional. One region of the molecule is concerned with binding to antigen while a different region mediates so-called effector functions. Effector functions include binding of the immunoglobulin to host tissues, to various cells of the immune system, to some phagocytic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

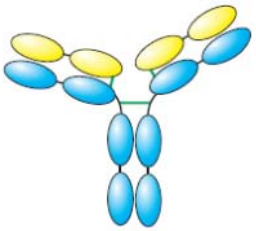
Surface and secreted antibodies



The B-cell antigen receptor (left) consists of two identical heavy (H) chains and two identical light (L) chains. In addition, secondary components (Ig-α and Ig-β) are closely associated with the primary receptor and are thought to couple it to intracellular signalling pathways. Circulating antibodies (right) are structurally identical to the primary B-cell antigen receptors, except that they lack the transmembrane and intracytoplasmic sections. Many proteolytic enzymes cleave antibody molecules into three fragments – two identical Fab (antigen binding) fragments and one Fc (crystallizable) fragment.

The Immune System



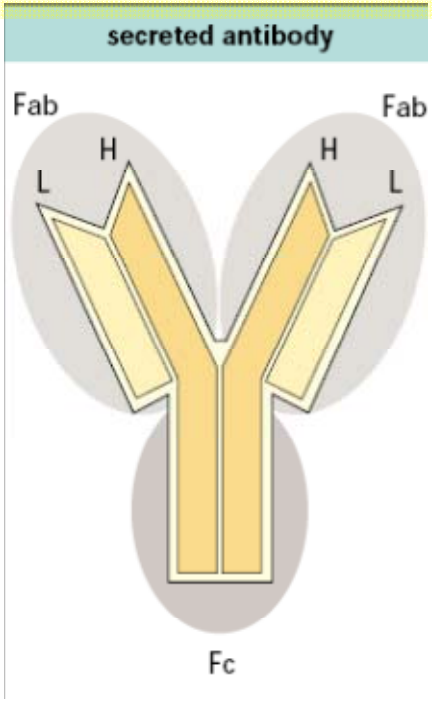


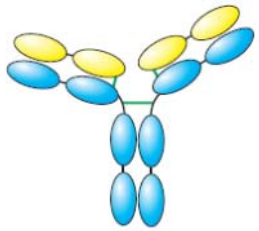
Antibodies

Immunoglobulin class and subclass depends on the structure of the heavy chain

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 are determined by its heavy chain type. Thus the four human IgG subclasses (IgG1, IgG2, IgG3 and IgG4) have heavy chains called γ1, γ2, γ3 and γ4 that differ slightly, although all are recognizably γ heavy chains.

The four subclasses of human IgG (IgG1–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 or IgE. This range of immunoglobulin class and subclass reflects isotypic variations in the immunoglobulin genes (see below). Immunoglobulin subclasses appear to have arisen late in evolution. Thus, the human IgG subclasses are very different from the four known subclasses of IgG that have been identified in the mouse. 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 *Figure. 4.3.*



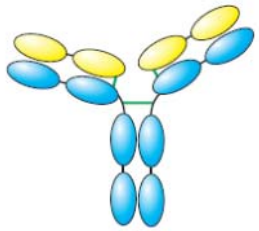


Antibodies

Physicochemical properties of human immunoglobulin classes

property	immunoglobulin type									
	IgG1	IgG2	IgG3	IgG4	IgM	IgA1	IgA2	sIgA	IgD	IgE
heavy chain	γ_1	γ_2	γ_3	γ_4	μ	α_1	α_2	α_1/α_2	δ	ϵ
mean serum conc. (mg/ml)	9	3	1	0.5	1.5	3.0	0.5	0.05	0.03	0.00005
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. 4.3 Each immunoglobulin class has a characteristic type of heavy chain. Thus IgG posesses γ chains; IgM, μ chains; IgA, α chains; IgD, δ chains; and IgE, ε 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 (s-IgA) in association with a protein chain termed the secretory component. The serum concentration of s-IgA is very low, whereas the level in intestinal secretions can be very high.



Antibodies

IgG – The major immunoglobulin in normal human serum, accounting for 70–75% of the total immunoglobulin pool, IgG consists of a single four-chain molecule with a sedimentation coefficient of 7S and a molecular weight of 146 000. However, IgG3 proteins are slightly larger than the other subclasses; due to the slightly heavier γ3 chain.

IgM – Accounts for approximately 10% of the immunoglobulin pool. The molecule is a pentamer of the basic four-chain structure. The individual heavy chains have a molecular weight of approximately 65 000 and the whole molecule has a molecular weight of 970 000.

IgD – Accounts for less than 1% of the total plasma immunoglobulin but is a major component of the surface membrane of many B cells.

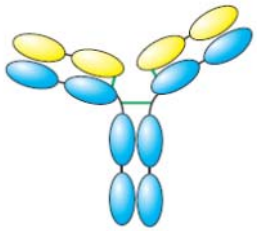
IgE – Though scarce in serum, is found on the surface membrane of basophils and mast cells in all individuals.

IgA – Represents 15–20% of the human serum immunoglobulin pool. In humans more than 80% of IgA occurs as a monomer of the four-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. Secretory IgA (s-IgA), may be of either subclass (IgA1 or IgA2), exists mainly in the 11S, dimeric form and has a molecular weight of 385 000 due to its association with another protein, known as the secretory component.

All light chains have one variable and one constant region

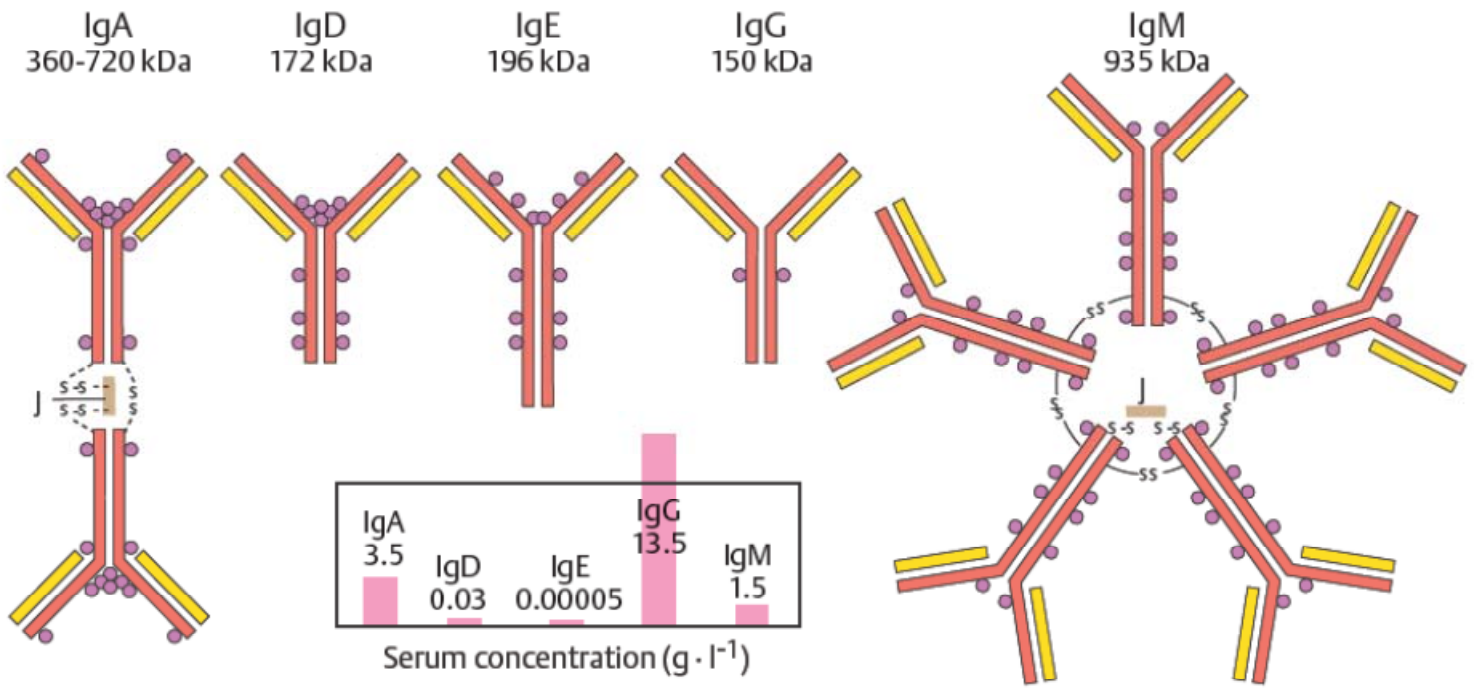
The light chains of most vertebrates have been shown to exist in two distinct forms called kappa (κ) and lambda (λ). These are isotypes, being present in all individuals. Either of the light chain types may combine with any of the heavy chain types, but in any one immunoglobulin molecule both light chains and both heavy chains are of the same type.

Hilschmann, 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 *Fig. 4.27*) 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.



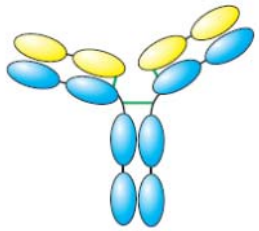
Antibodies

B. Classes of immunoglobulins



Chain:					
H	α	δ	ε	γ	μ
L	κ or λ	κ or λ	κ or λ	κ or λ	κ or λ
Structure:					
	$(\alpha_2 \kappa_2)_n J$	$\delta_2 \kappa_2$	$\varepsilon_2 \kappa_2$	$\gamma_2 \kappa_2$	$(\mu_2 \kappa_2)_5 J$
	$(\alpha_2 \lambda_2)_n J$	$\delta_2 \lambda_2$	$\varepsilon_2 \lambda_2$	$\gamma_2 \lambda_2$	$(\mu_2 \lambda_2)_5 J$

n = 1, 2 or 3



Antibodies

The basic structure of IgG1

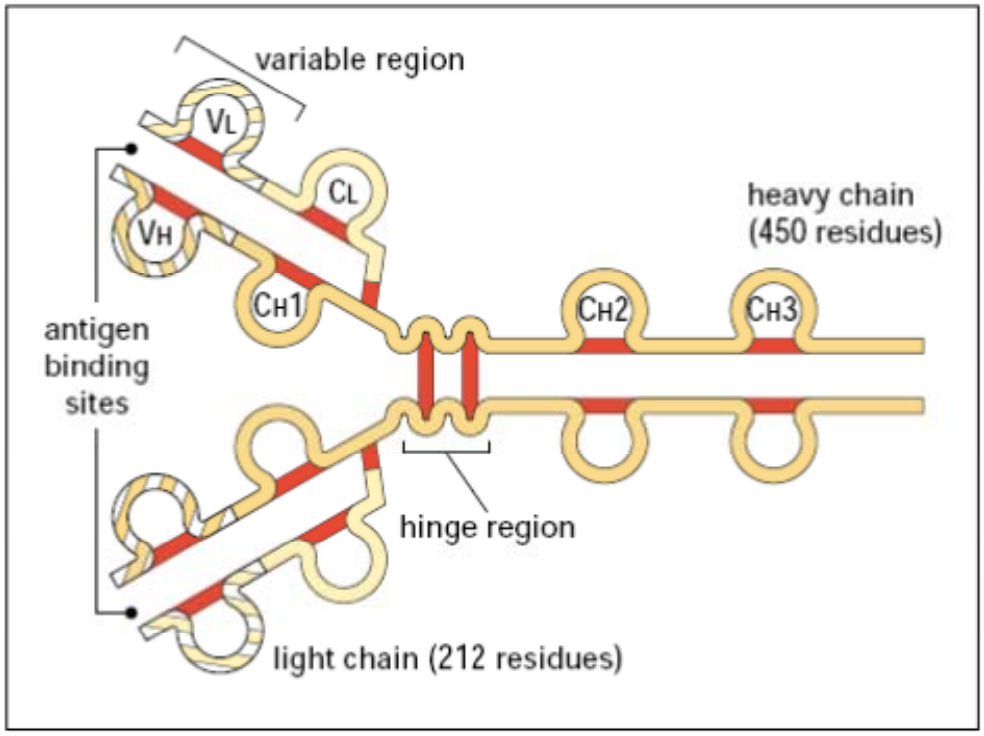
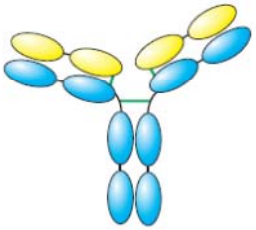
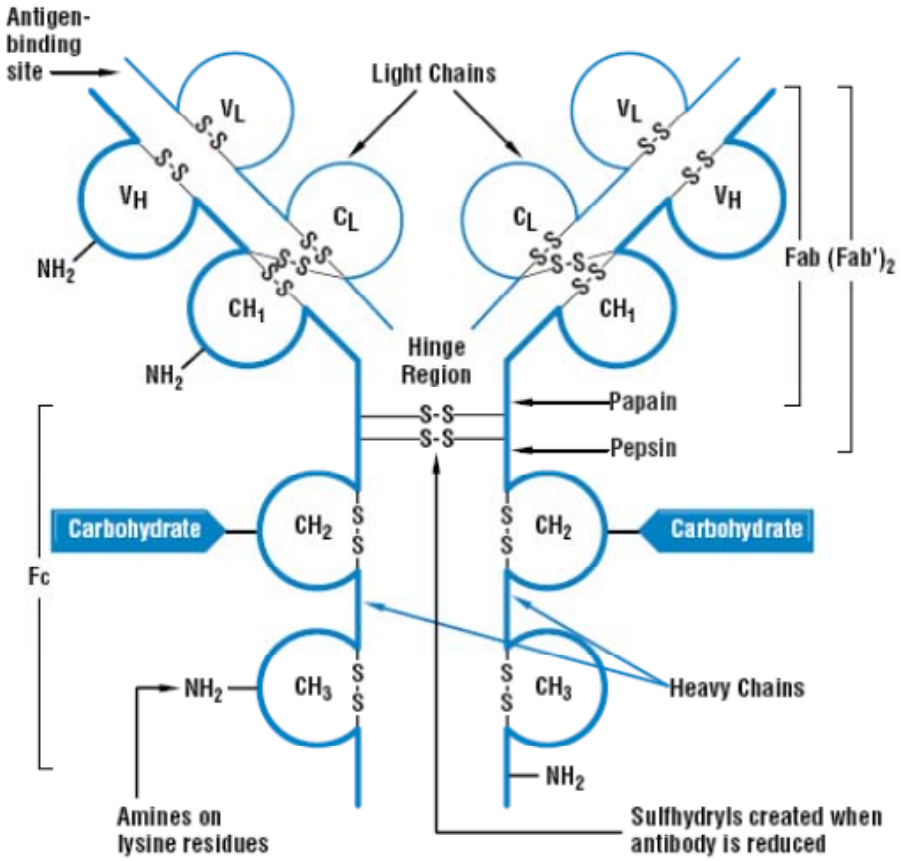
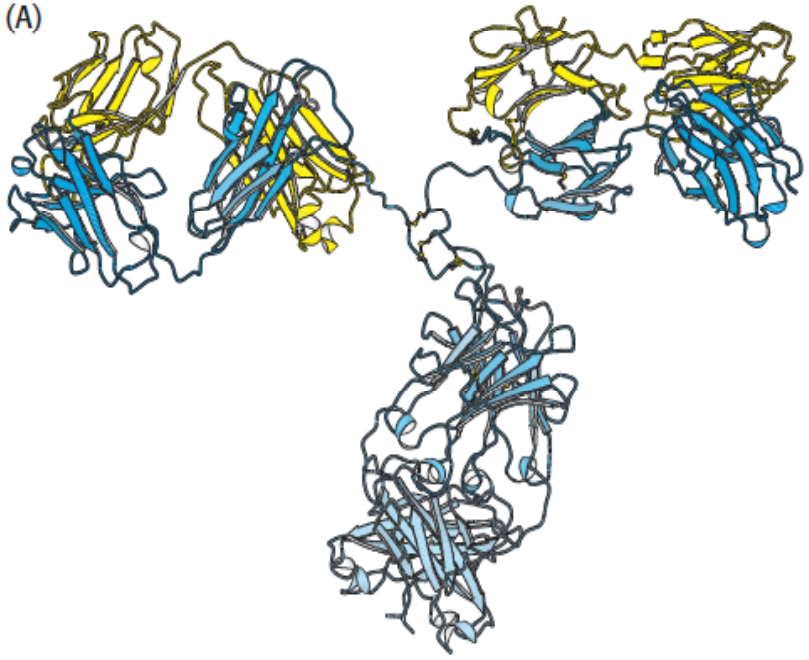


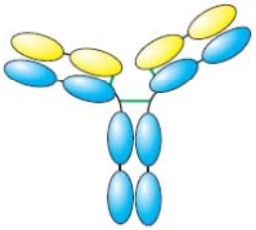
Fig. 4.4 The N-terminal end of IgG1 is characterized by sequence variability (V) in both the heavy and light chains, referred to as the V_H and V_L regions respectively. The rest of the molecule has a relatively constant (C) structure. The constant portion of the light chain is termed the C_L region. The constant portion of the heavy chain is further divided into three structurally discrete regions: C_H1, C_H2 and C_H3. 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 C_H1 and C_H2 domains. Flexibility in this area permits the two antigen-binding sites to operate independently. There is close pairing of the domains except in the C_H2 region (see *Fig. 4.6*). Carbohydrate moieties are attached to the C_H2 domains.



Antibodies


Antigen-binding site
Light Chains
V_L
V_H
C_L
NH_2
CH_1
Hinge Region
Papain
Pepsin
Fab (Fab')$_2$
Carbohydrate
CH_2
Fc
CH_3
Heavy Chains
Amines on lysine residues
Sulfhydryls created when antibody is reduced


(A)



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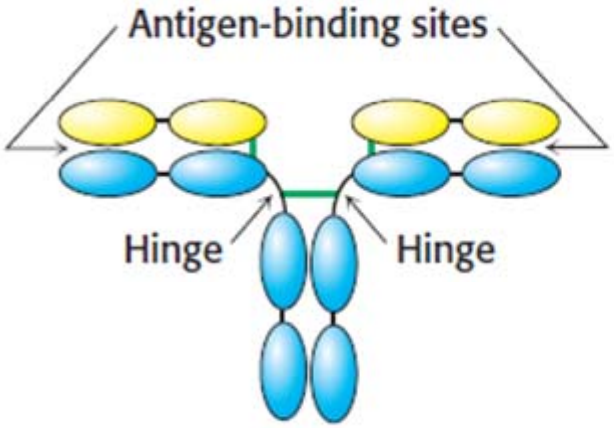
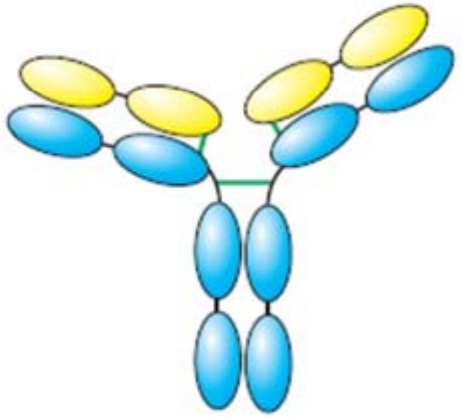
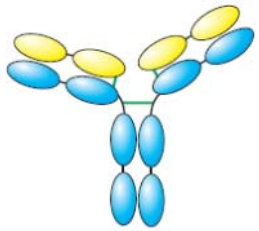




Segmental flexibility. The linkages between the F_{ab} and the F_c regions of an IgG molecule are flexible, allowing the two antigen-binding sites to adopt a range of orientations with respect to one another. This flexibility allows effective interactions with a multivalent antigen without requiring that the epitopes on the target be a precise distance apart.



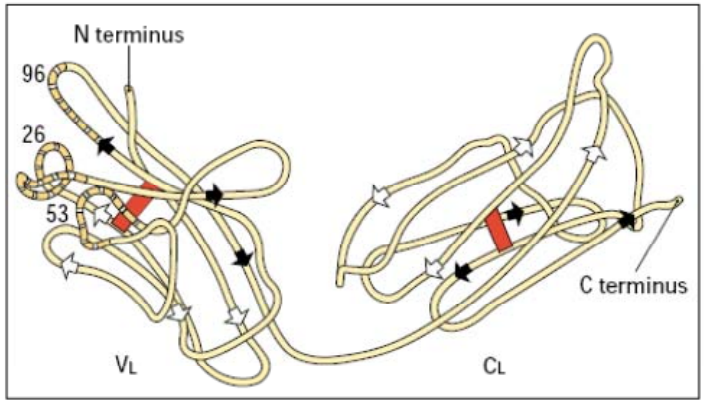
Antibodies

IgG has a 'typical' antibody structure

The IgG molecule may be thought of as a 'typical' antibody (Fig. 4.4). It has two intrachain disulphide bonds in the light chain – one in the variable region and one in the constant region (*Fig. 4.5*). There are four such bonds in the heavy (γ) chain, which is twice the length of the light chain. Each disulphide bond encloses a peptide loop of 60–70 amino acid residues; 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 *Figure 4.5*.

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

Basic folding in the light chain



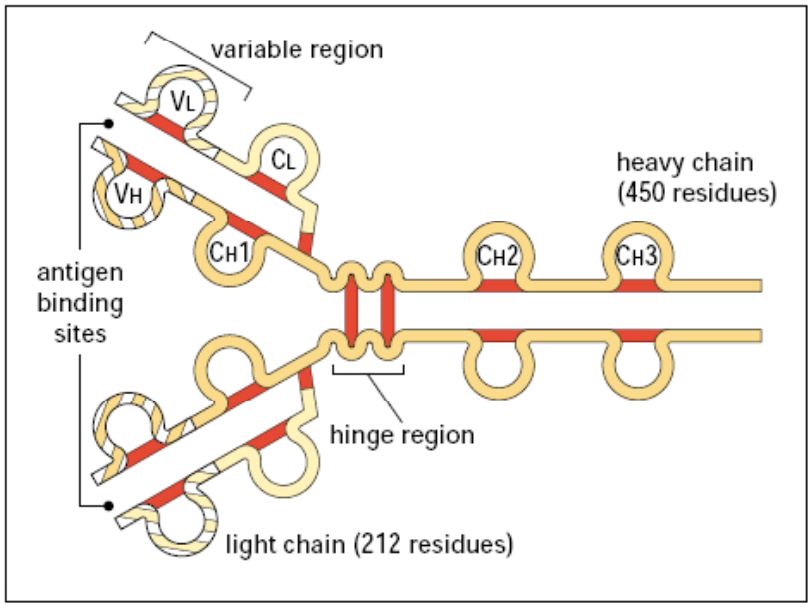
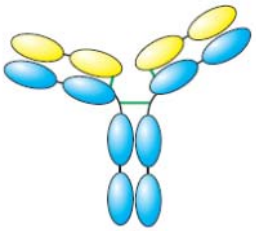
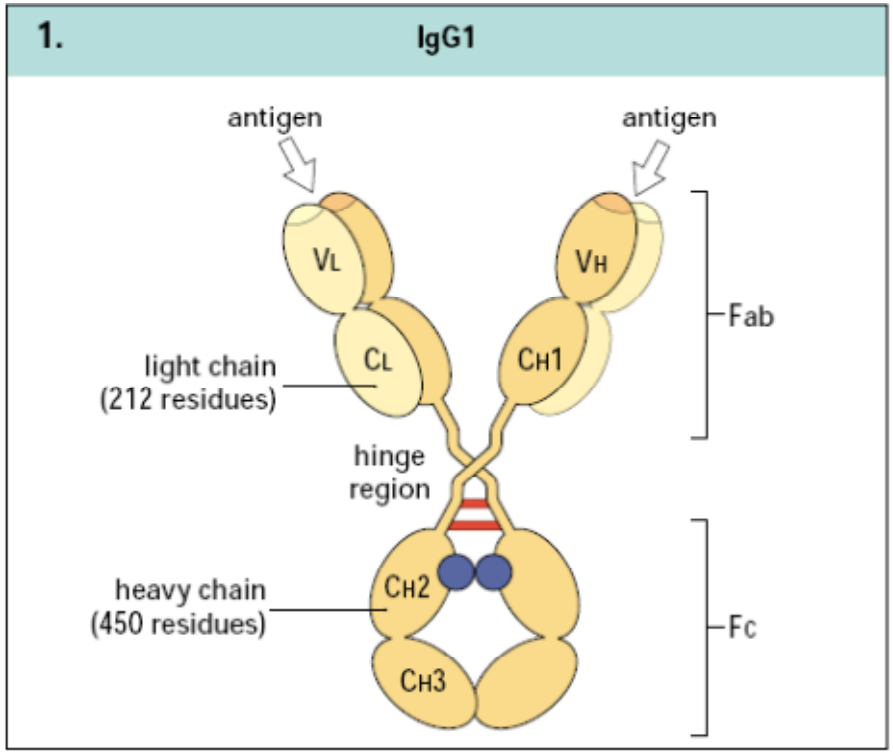


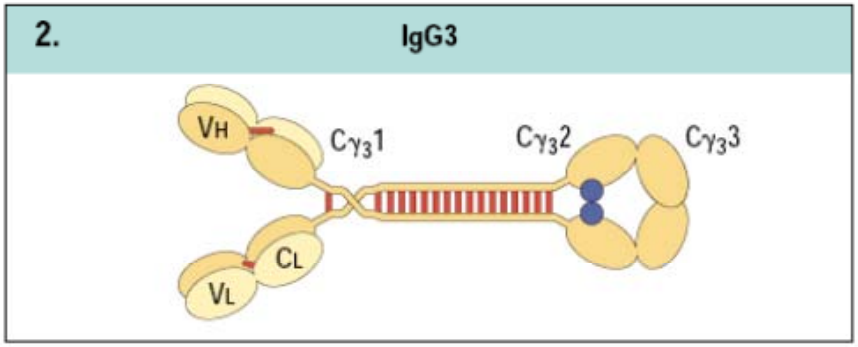
Fig. 4.5 The immunoglobulin domains in the light chain share a basic folding pattern with several straight segments of polypeptide chain lying parallel to the long axis of the domain. Light chains have two domains – one constant and one variable. Within each domain, the polypeptide chain is arranged 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 bond (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.

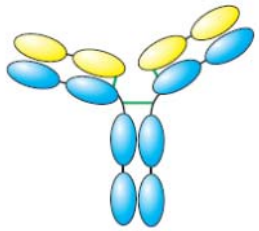


Antibodies

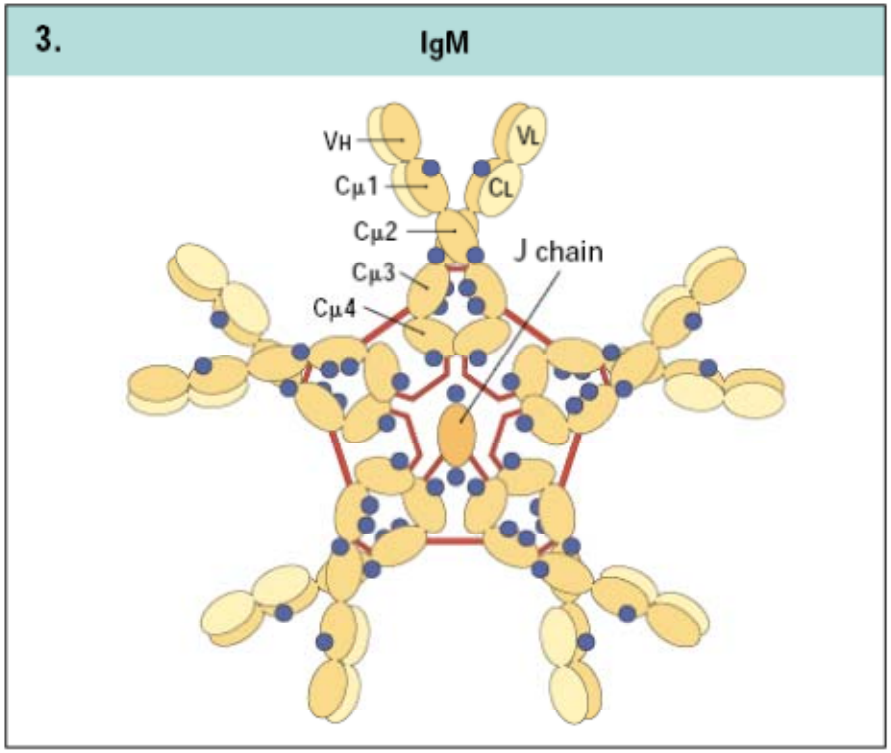
IgG – With human IgG, the four subclasses differ only slightly in their amino acid sequences. Most of the differences are clustered in the hinge region and give rise to differing patterns of interchain disulphide bonds between the four proteins. The most striking structural difference is the elongated hinge region of IgG3, which accounts for its higher molecular weight and possibly for some of its enhanced biological activity (*Fig. 4.6(2)*).







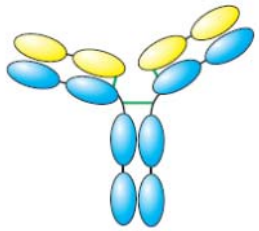
Antibodies



IgM – Human IgM is usually found as a pentamer of the basic four-chain unit (*Fig. 4.6(3)*). The μ chains of IgM differ from γ chains in amino acid sequence and have an extra constant region domain. The subunits of the pentamer are linked by disulphide bonds between the Cμ3 domains, and possibly by disulphide bonds between the C-terminal 18-residue peptide tailpieces. The complete molecule consists of a densely packed central region with radiating arms, as seen in electron micrographs.

Photographs of IgM antibodies binding to bacterial flagella show molecules adopting a 'crab-like' configuration (*Fig. 4.8*). This suggests that flexion readily occurs between the Cμ2 and Cμ3 domains, although note that this region is not structurally homologous to the IgG hinge. The dislocation resulting in the 'crab-like' 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 μ 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 an Ig-like domain 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, there is evidence that hexameric IgM becomes the preferred form.



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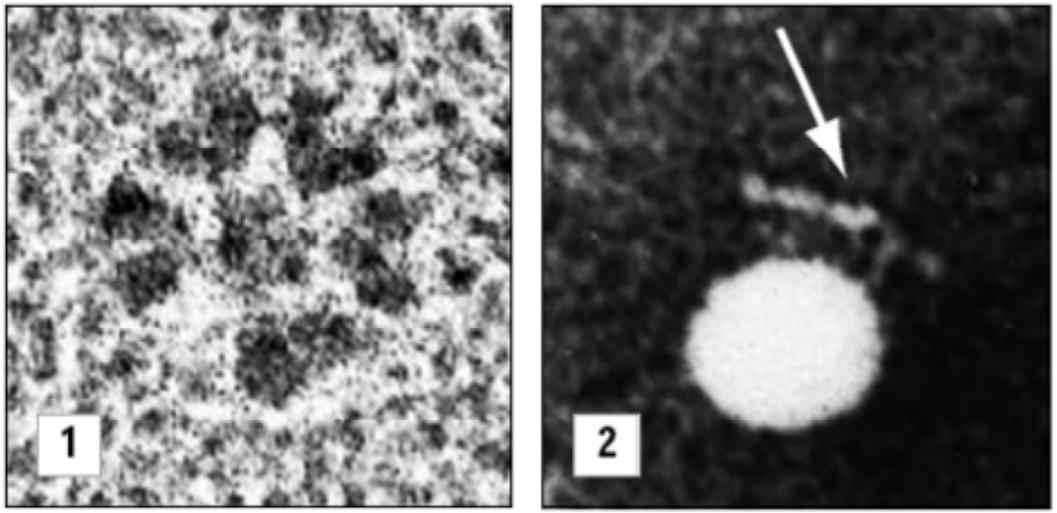
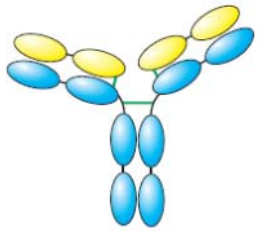


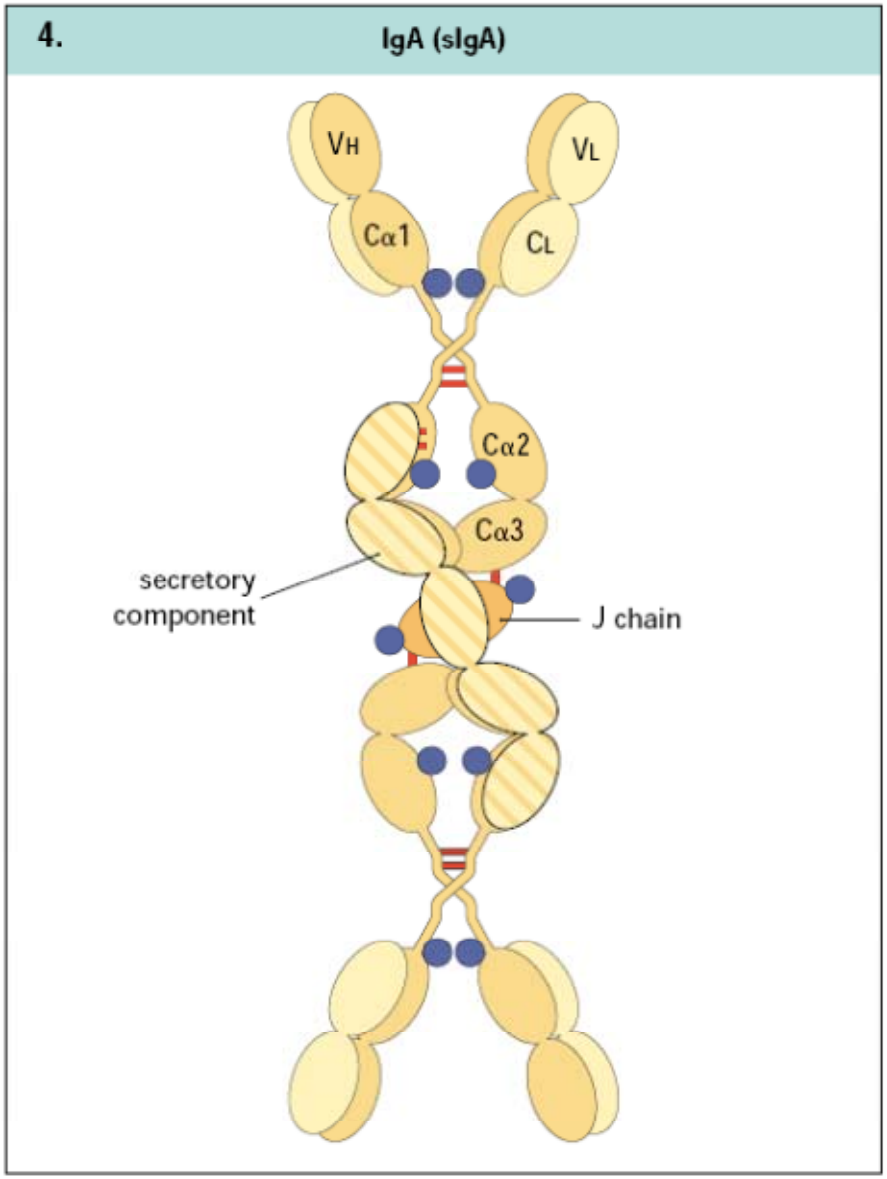
Electron micrographs of IgM molecules. (**1**) In free solution, deer IgM adopts the characteristic star-shaped configuration. ×195 000. (Courtesy of Drs E. Holm Nielson P. Storgaard and Prof S-E. Svehag.) (**2**) Rabbit IgM antibody (arrowed) in 'crab-like' configuration with partly visible central ring structure bound to a poliovirus virion ×190 000. (Courtesy of Dr B. Chesebro and Professor S-E Svehag.)

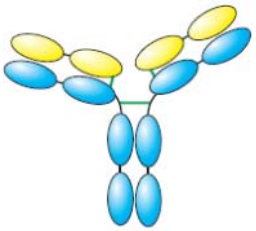


Antibodies

IgA – The 472 amino acid residues of the α chain are arranged in four domains: VH, Cα1, Cα2 and Cα3. A feature shared with IgM is an additional C-terminal 18-residue peptide with a penultimate cysteine residue, which is able to bind covalently to a J chain to form dimers. Electron micrographs of IgA dimers show double Y-shaped structures, suggesting that the monomeric subunits are linked end-to-end through the C-terminal Cα3 regions (*Fig. 4.9*).

Secretory IgA (s-IgA) exists mainly in the form of a molecule sedimenting at 11S (mol. wt 380 000). The complete molecule is made up of two units of IgA, one secretory component (mol. wt 70 000) and one J chain (mol. wt 15 000) (*Fig. 4.6(4)*). It is not clear how the various peptide chains are linked together. In contrast to the J chain, secretory component is not synthesized by plasma cells but by epithelial cells. IgA held in dimer configuration by a J chain, and secreted by submucosal plasma cells, actively binds secretory component as it traverses epithelial cell layers. Bound secretory component facilitates the transport of s-IgA into secretions, as well as protecting it from proteolytic attack.





Antibodies

Transport of IgA across the mucosal epithelium

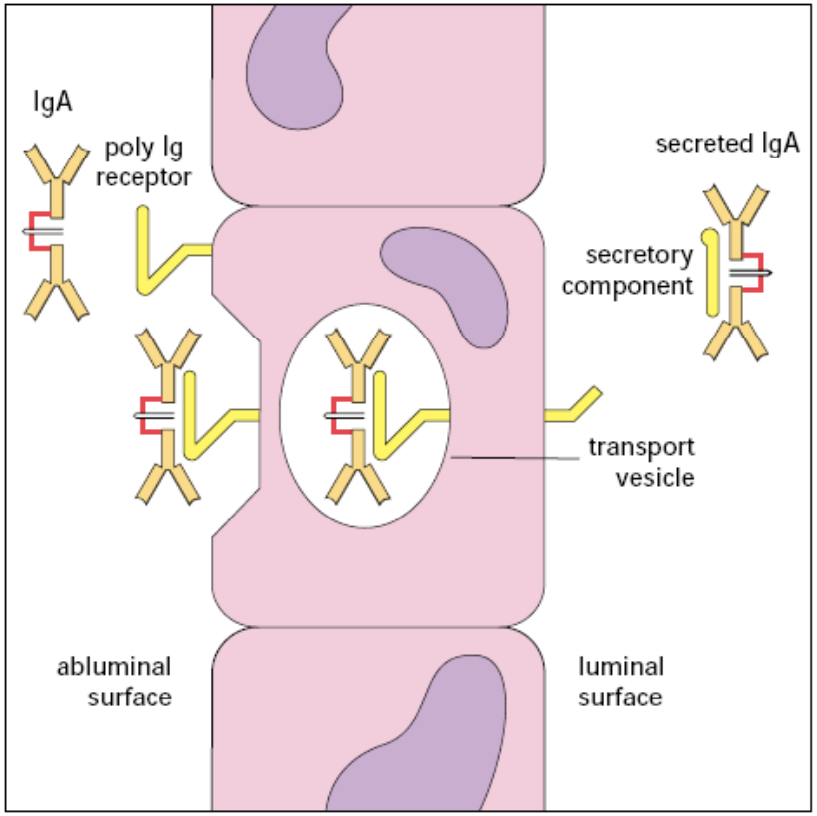
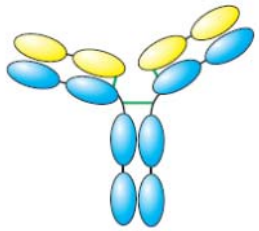


Fig. 4.21 IgA dimers secreted into the intestinal lamina propria by plasma cells bind to poly-Ig receptors on the internal (abluminal) surface of the epithelial cells. The s-IgA–receptor complex is then endocytosed and transported across the cell while still bound to the membrane of transport vesicles. These vesicles fuse with the plasma membrane at the luminal surface, releasing IgA dimers with bound secretory component derived from cleavage of the receptor. The dimeric IgA is protected from proteolytic enzymes in the lumen by the presence of this secretory component.



Antibodies

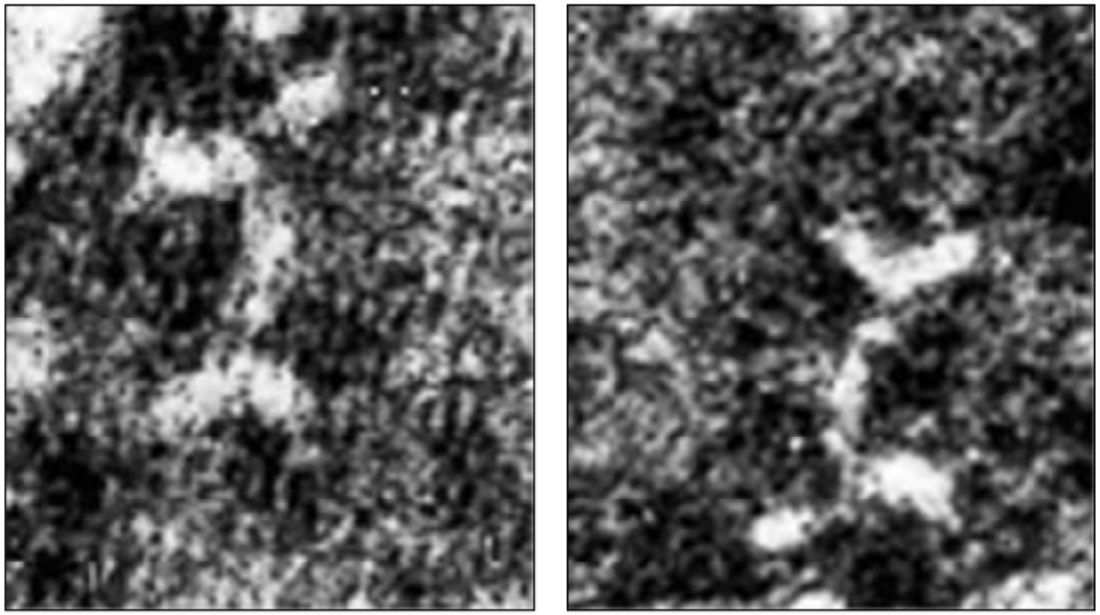
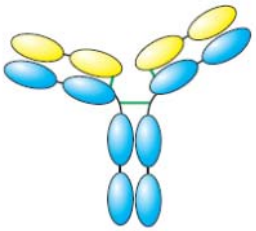
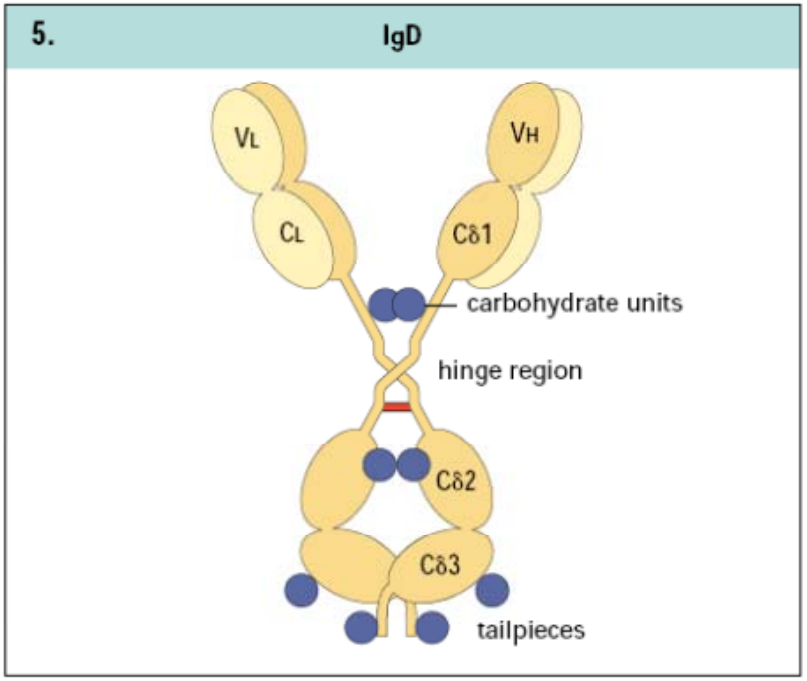


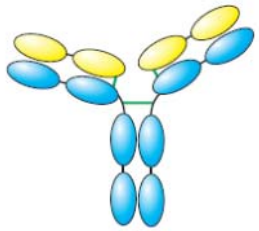
Fig. 4.9 Electron micrographs of human dimeric IgA molecules. The double Y-shaped appearance suggests that the monomeric subunits are linked end to end through the C-terminal Cα3 domain ×250 000. (Courtesy of Professor S-E. Svehag.)



Antibodies

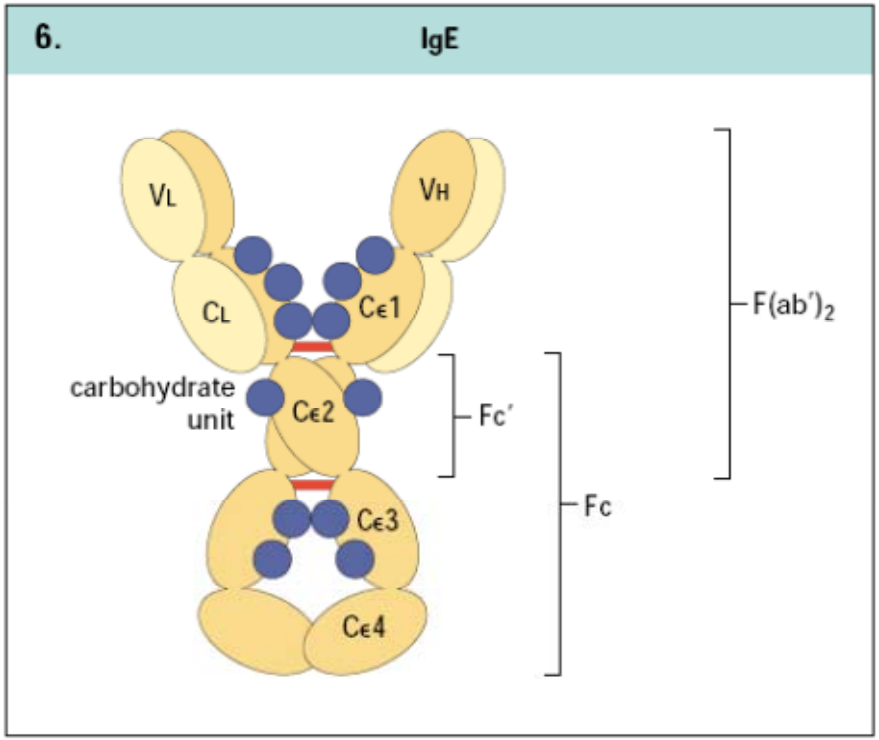
IgD – Less than 1% of the total immunoglobulin in serum is IgD. This protein is more susceptible to proteolysis than IgG1, IgG2, IgA or IgM, and also has a tendency to undergo spontaneous proteolysis. There appears to be a single disulphide bond between the δ chains and a large amount of carbohydrate distributed in multiple oligosaccharide units (*Fig. 4.6(5)*).

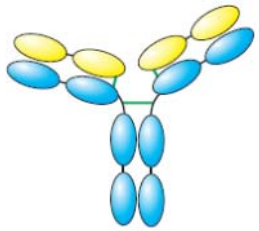




Antibodies

IgE – The structure of IgE is shown in *Figure 4.6(6)*. The higher molecular weight of the ε chain (72 500) is explained by the larger number of amino acid residues (approximately 550) distributed over five domains (VH, Cε1, Cε2, Cε3 and Cε4).





Antibodies

INTERACTION OF ANTIBODIES WITH ANTIGENS

Antibodies form multiple non-covalent bonds with antigen

X-ray crystallography studies of antibody V domains show that the hypervariable regions are clustered at the end of the Fab arms; particular residues in these regions interact specifically with antigen. The framework residues do not usually form bonds with the antigen. However, they are essential for producing the folding of the V domains and maintaining the integrity of the binding site. The binding of antigen to antibody involves the formation of multiple non-covalent bonds between the antigen and amino acids of the binding site. Considered individually, the attractive forces (hydrogen and electrostatic bonds, Van der Waals and hydrophobic forces) are weak by comparison with covalent bonds. However, the large number of interactions results in a large total binding energy.

Intermolecular attractive forces

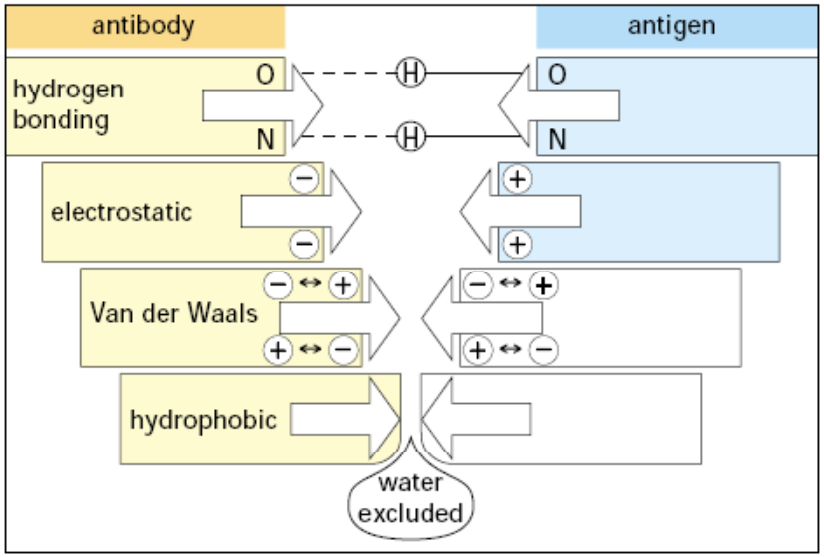
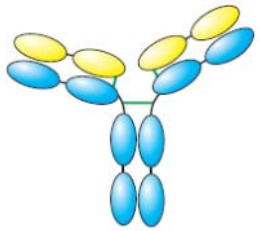


Fig. 4.10 The forces binding antigen to antibody require the close approach of the interacting groups. Hydrogen bonding results from the formation of hydrogen bridges between appropriate atoms. Electrostatic forces derive from the attraction of oppositely charged groups located on two protein side-chains. Van der Waals bonds are generated by the interaction between electron clouds (here represented as induced oscillating dipoles). Hydrophobic bonds (which may contribute up to half the total strength of the antigen–antibody bond) rely on the association of non-polar, hydrophobic groups so that contact with water molecules is minimized. The distance between the interacting groups that gives optimum binding depends on the type of bond.



Antibodies

The conformations of target antigen and binding site are complementary

The strength of a non-covalent bond is critically dependent on the distance (d) between the interacting groups. The force is proportional to $1/d^2$ for electrostatic forces, and to $1/d^7$ for Van der Waals forces. Thus the interacting groups must be close (in molecular terms) before these forces become significant (*Fig. 4.10*). In order for an antigenic determinant (epitope) and an antibody-combining site (paratope) to combine (*Fig. 4.11*), there must be suitable atomic groupings on opposing parts of the antigen and antibody, and the shape of the combining site must fit the epitope, so that several non-covalent bonds can form simultaneously. If the antigen and the combining site are complementary in this way, there will be sufficient binding energy to resist thermodynamic disruption of the bond. However, if electron clouds of the antigen and antibody overlap, steric repulsive forces come into play which are inversely proportional to the 12th power of the distance between the clouds: $F \propto 1/d^{12}$. These forces have a vital role in determining the specificity of the antibody molecule for a particular antigen, and its ability to discriminate between antigens, as any variation from the ideal complementary shape will cause a decrease in the total binding energy through increased repulsive forces and decreased attractive forces (*Fig. 4.11*). This is not to say that the antigen-binding site is completely inflexible; when antigen binds to antibody individual amino acid residues may become slightly displaced from their position in the free state. This is referred to as 'induced fit', but it will only occur when the energy gain in the overall antigen–antibody bond offsets that needed to induce the fit.

Good fit and poor fit

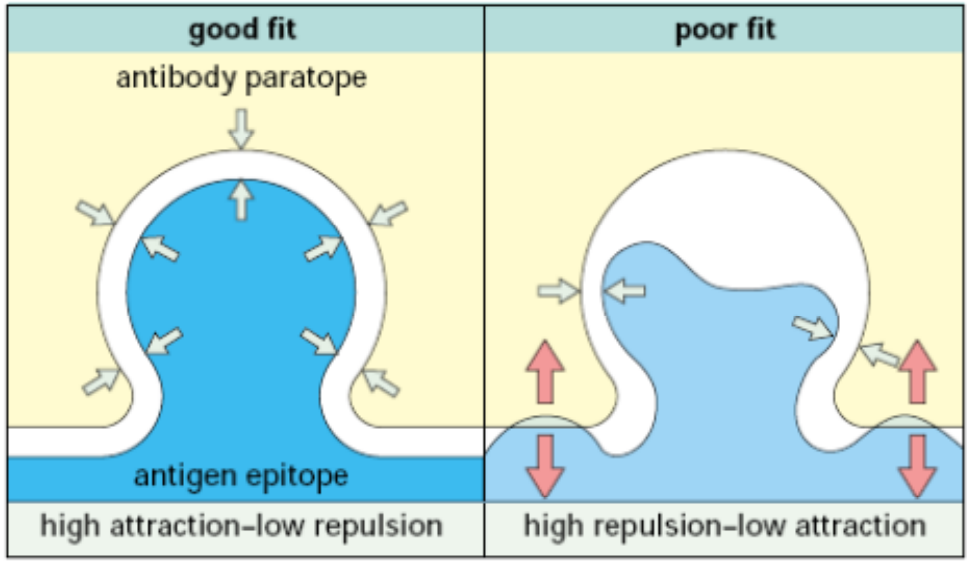
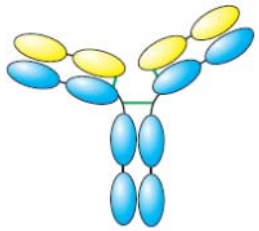


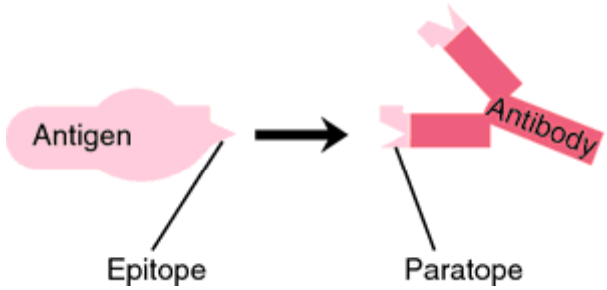
Fig. 4.11 A good fit between the antigenic determinant and the binding site of the antibody will create ample opportunities for intermolecular attractive forces to be created and few opportunities for repulsive forces to operate. Conversely, when there is a poor fit, the reverse is true. When electron clouds overlap, high repulsive forces are generated which override any small forces of attraction.

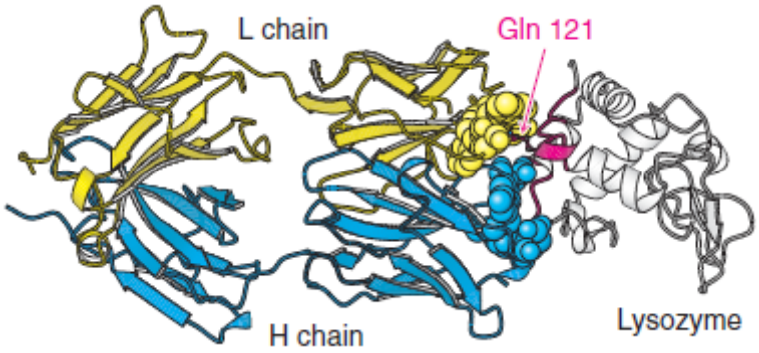


Glossary

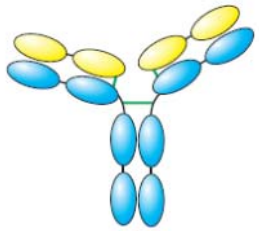
An **epitope**, also known as **antigenic determinant**, is the part of an antigen that is recognized by the immune system, specifically by antibodies, B cells, or T cells.

The **paratope** is the part of an antibody which recognizes an antigen, the antigen-binding site of an antibody. It is a small region (of 15–22 amino acids) of the antibody's Fab region and contains parts of the antibody's heavy and light chains.





Antibody–protein interactions. The structure of a complex between an F_{ab} fragment and lysozyme reveals that the binding surfaces are complementary in shape over a large area. A single residue of lysozyme, glutamine 121, penetrates more deeply into the antibody combining site.



Antibodies

The conformations of target antigen and binding site are complementary

Antibody affinity indicates the strength of a single antigen–antibody bond

The strength of the bond between an antigen and an antibody is known as the antibody affinity. It is the sum of the attractive and repulsive forces described above (*Fig. 4.13*). Interaction of the antibody-combining site with antigen can be investigated thermodynamically. To measure the affinity of a single combining site, it is necessary to use a monovalent antigen, or even a single isolated antigenic determinant (a hapten). Because the non-covalent bonds between antibody and epitope are dissociable, the overall combination of an antibody and antigen must be reversible; thus the Law of Mass Action can be applied to the reaction and the equilibrium constant, K, can be determined. This is the affinity constant (*Fig. 4.14*).

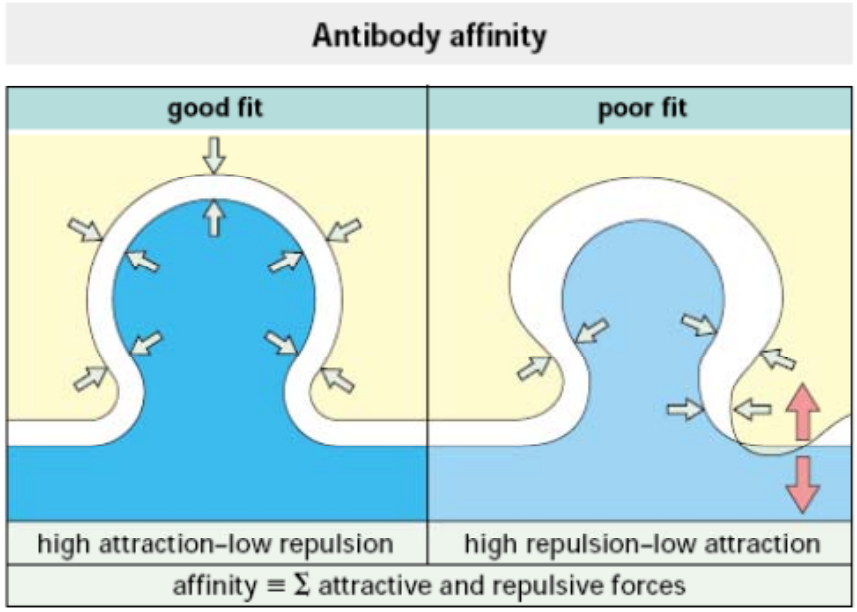


Fig. 4.13 The affinity with which antibody binds antigen is the sum of the attractive and repulsive forces between them. A high-affinity antibody implies a good fit and, conversely, a low-affinity antibody implies a poor fit.

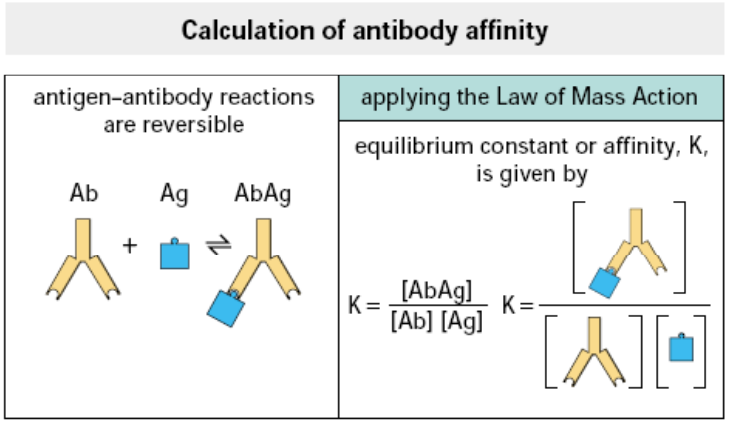
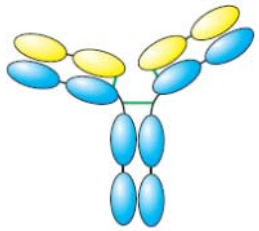


Fig. 4.14 All antigen–antibody reactions are reversible. The Law of Mass Action can therefore be applied, and the antibody affinity (given by the equilibrium constant, K) can be calculated. (Square brackets refer to the concentrations of the reactants.)



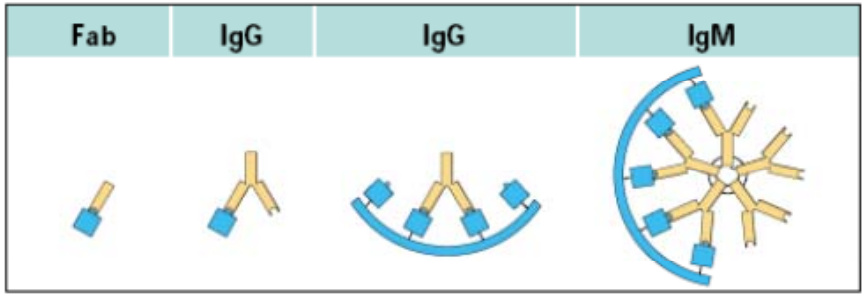
Antibodies

The conformations of target antigen and binding site are complementary

Antibody avidity indicates the overall strength of interaction between antibody and antigen

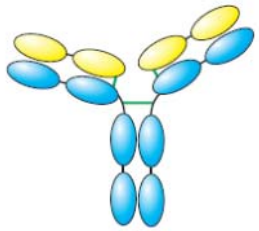
Because each antibody unit of four polypeptide chains has two antigen-binding sites, antibodies are potentially multivalent in their reaction with antigen. In addition, antigen can also be monovalent (e.g. haptens) or multivalent (e.g. microorganisms). The strength with which a multivalent antibody binds a multivalent antigen is termed avidity, to differentiate it from the affinity of a single antigenic determinant for an individual combining site. The avidity of an antibody for its antigen is dependent on the affinities of the individual combining sites for the determinants on the antigen. It is greater than the sum of these affinities if both antibody-binding sites can combine with the antigen. This is because all the antigen–antibody bonds must be broken simultaneously before the antigen and antibody dissociate (*Fig. 4.15*). In normal physiological situations, avidity is likely to be more relevant than affinity, as naturally occurring antigens are multivalent. However, the precise measurement of hapten–antibody affinity is more likely to give an insight into the immunochemical nature of the antigen–antibody reaction.

Affinity and avidity



antibody	Fab	IgG	IgG	IgM
effective antibody valence	1	1	2	up to 10
antigen valence	1	1	n	n
equilibrium constant (L/M)	10^4	10^4	10^7	10^{11}
advantage of multivalence	–	–	10^3-fold	10^7-fold
definition of binding	affinity	affinity	avidity	avidity
	intrinsic affinity		functional affinity	

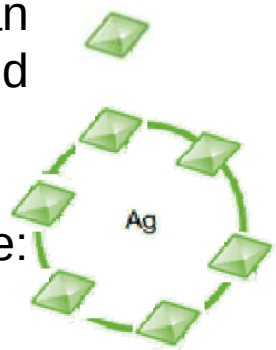
Fig. 4.15 Multivalent binding between antibody and antigen (avidity or functional affinity) results in a considerable increase in stability as measured by the equilibrium constant, compared with simple monovalent binding (affinity or intrinsic affinity, here arbitrarily assigned a value of 10^4 L/M^{-1}). This is sometimes referred to as the 'bonus effect' of multivalency. Thus there may be a 10^3-fold increase in the binding energy of IgG when both valencies (combining sites) are utilized and a 10^7-fold increase when IgM binds antigen in a multivalent manner.



Natural Antigen classification

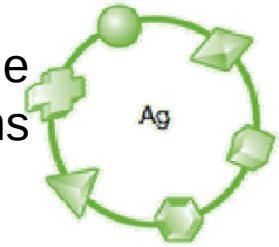
Antigens are classified according to their binding characteristics of valency (meaning the total number of sites) and their determinacy (meaning the number of different types of sites). There are four classes of antigens:

1. Only a single epitope on the surface that is capable of binding to an antibody: **unideterminate** and **univalent**. Haptens are unideterminate and univalent. NO NATURAL

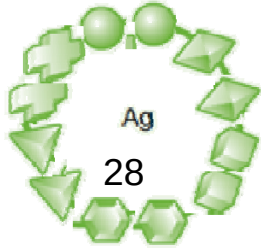


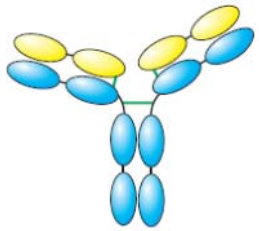
2. Two or more epitopes of the same kind on one antigen molecule: **unideterminate** and **multivalent**. NO NATURAL

3. Many epitopes of different kinds, but only one of each kind on one antigen molecule: **multideterminate** and **univalent**. Most protein antigens fall into this category.

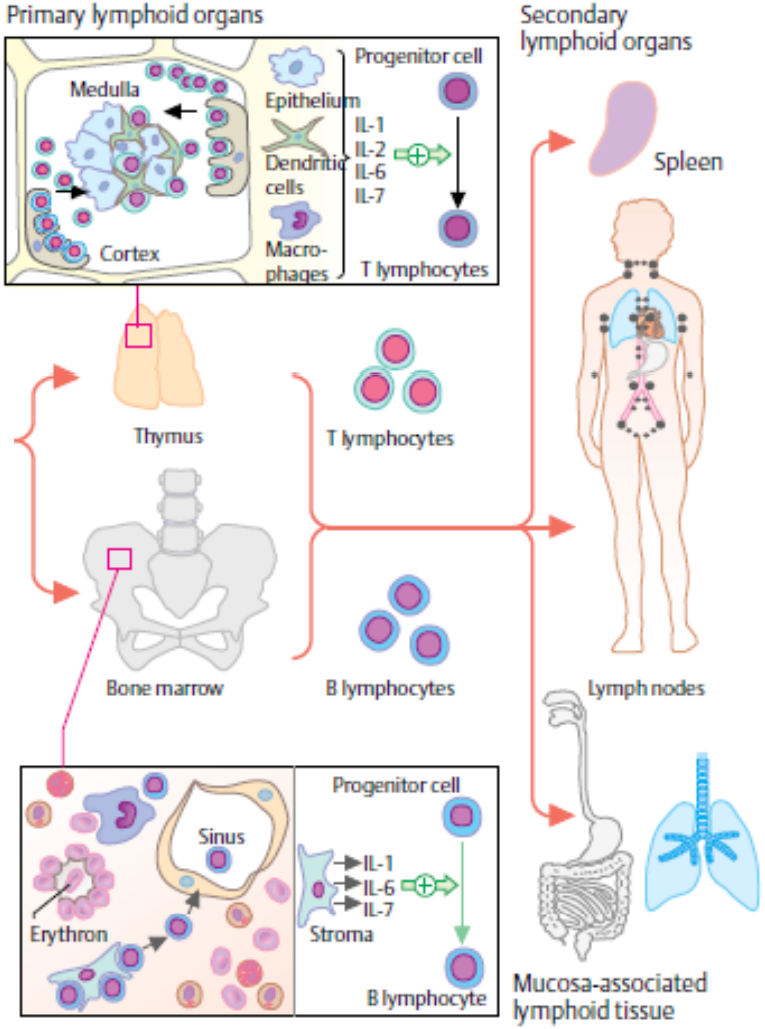


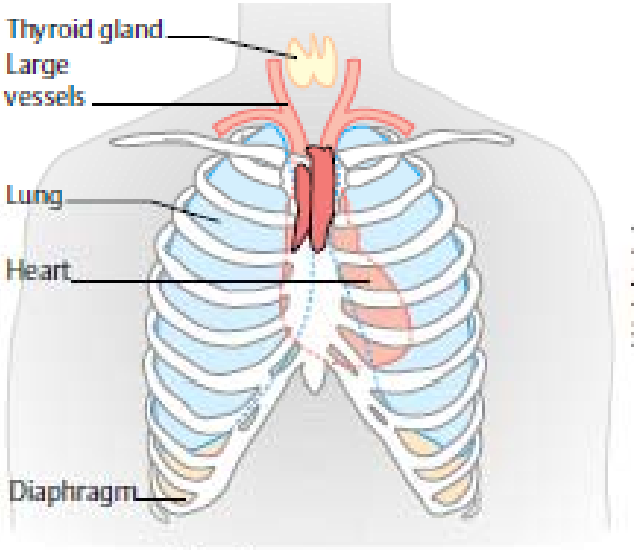
4. Many epitopes of different kinds, and more than one of each kind per antigen molecule: **multideterminate** and **multivalent**. Proteins containing multiple identical subunits, as well as polymerized proteins and whole cells, fall into this category.



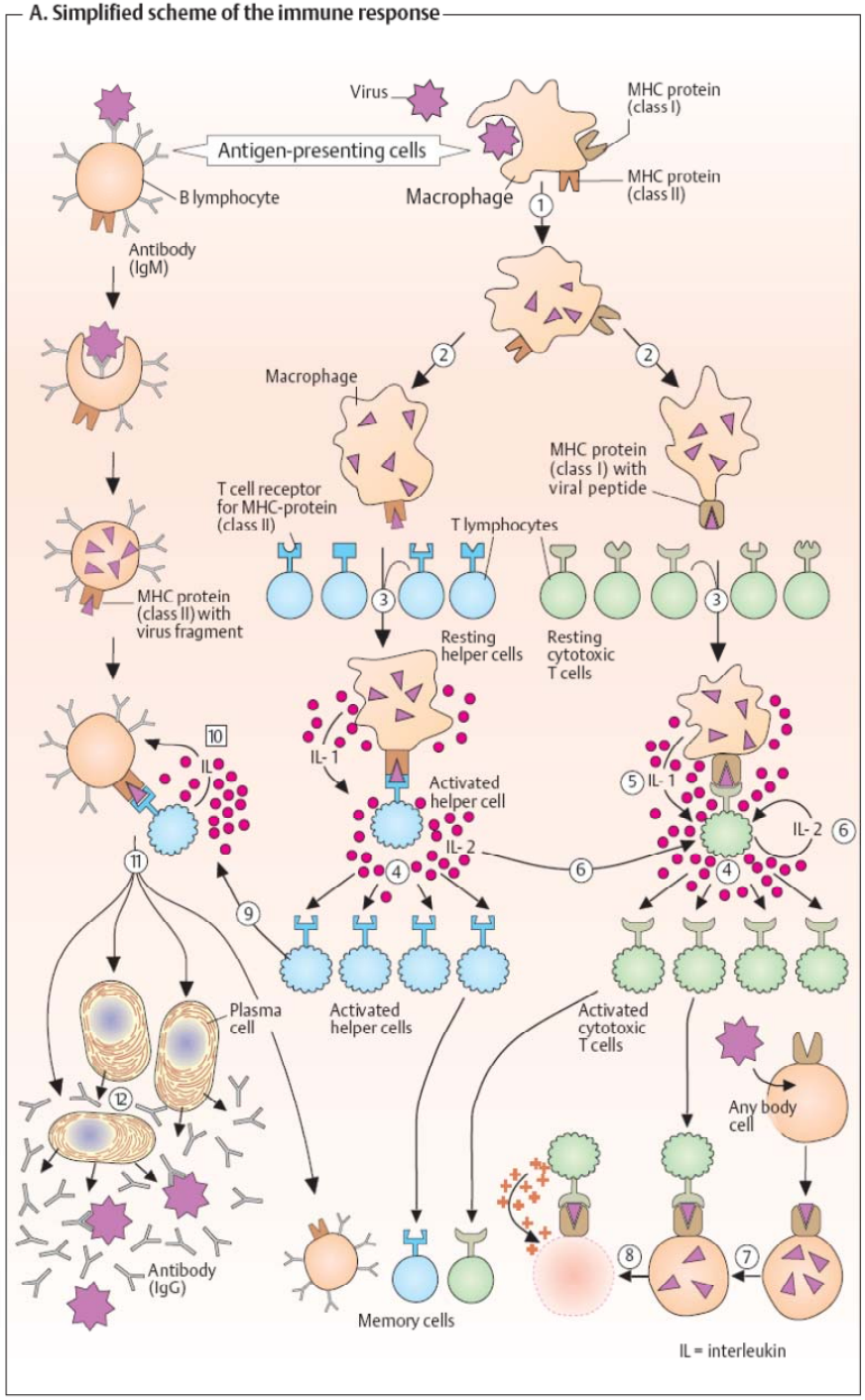


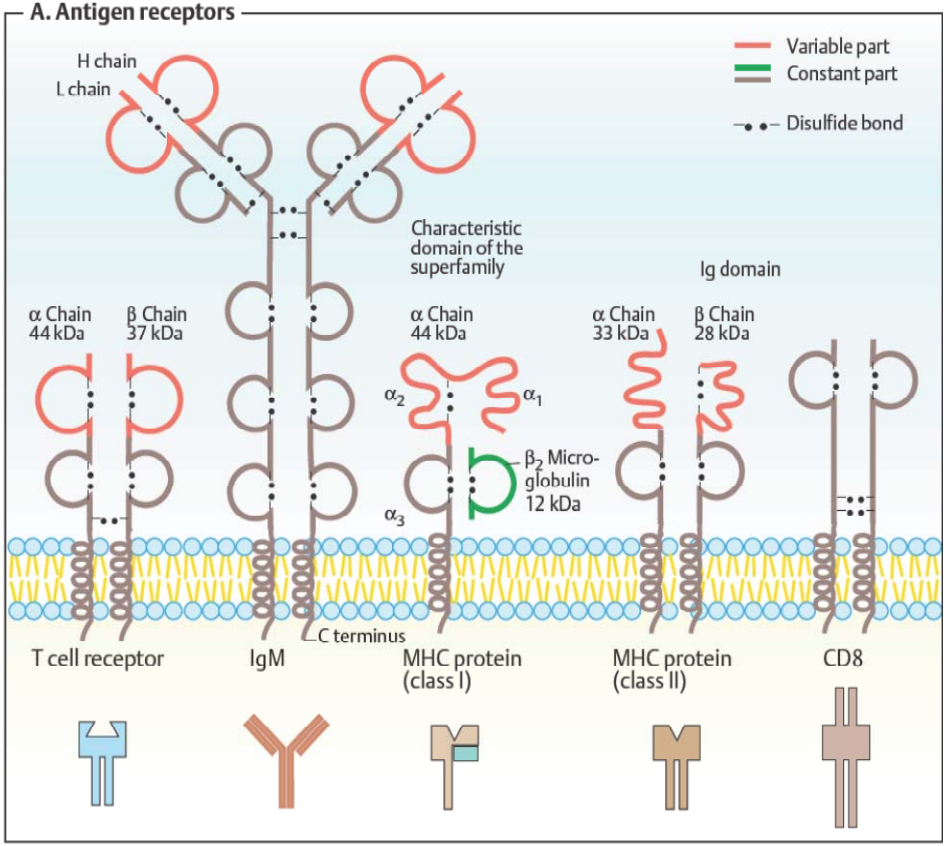
Lymphoid Organs



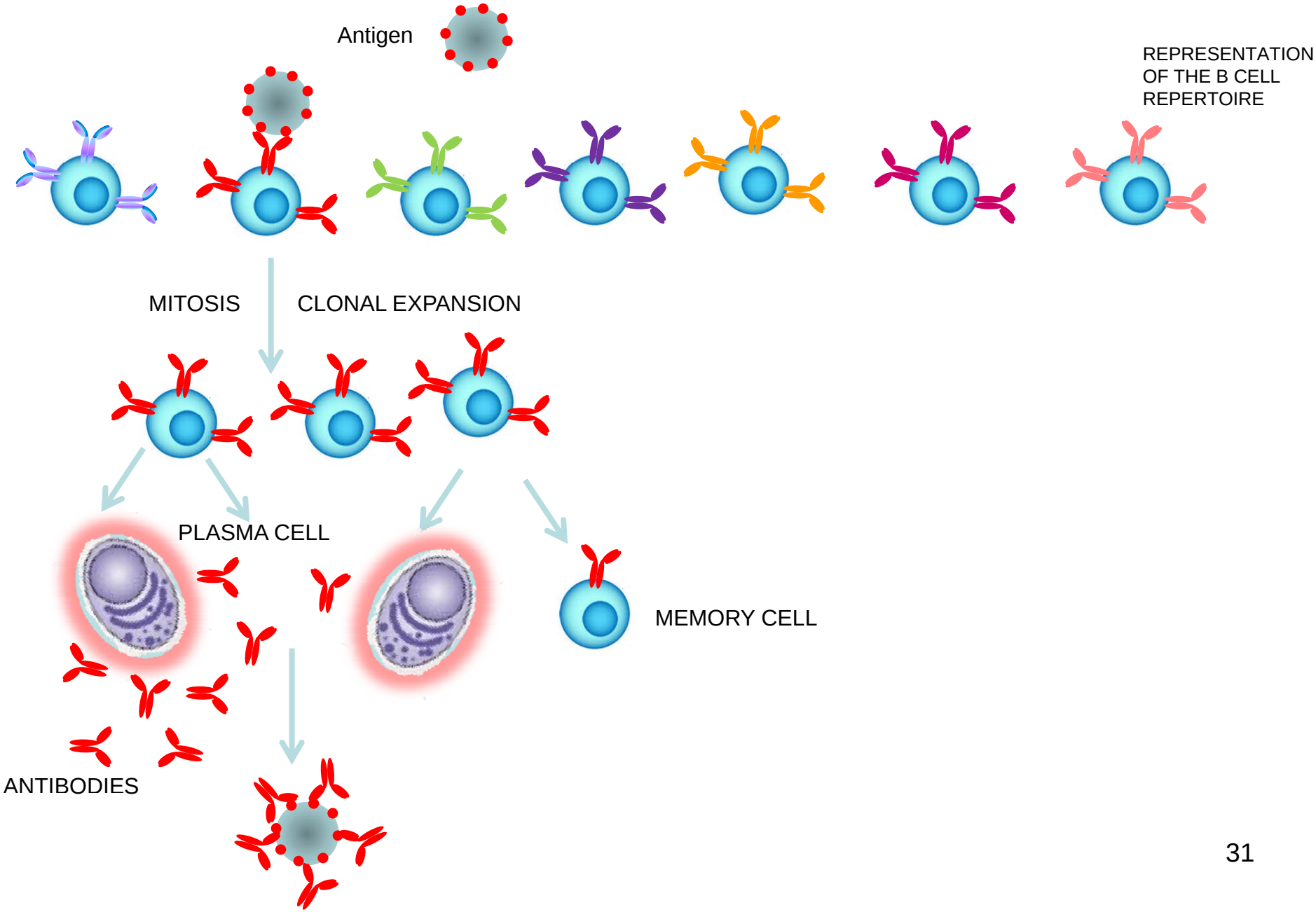


1. Position of the thymus


A. Simplified scheme of the immune response
Virus
MHC protein (class I)
Antigen-presenting cells
MHC protein (class II)
B lymphocyte
Macrophage
Antibody (IgM)
Macrophage
MHC protein (class I) with viral peptide
T cell receptor for MHC-protein (class II)
T lymphocytes
MHC protein (class II) with virus fragment
Resting helper cells
Resting cytotoxic T cells
IL-1
Activated helper cell
IL-2
IL-1
IL-2
Plasma cell
Activated helper cells
Activated cytotoxic T cells
Any body cell
Antibody (IgG)
Memory cells
IL = interleukin


A. Antigen receptors
Variable part
Constant part
Disulfide bond
H chain
L chain
Characteristic domain of the superfamily
Ig domain
α Chain 44 kDa
β Chain 37 kDa
α Chain 44 kDa
α Chain 33 kDa
β Chain 28 kDa
β2 Micro-globulin 12 kDa
C terminus
T cell receptor
IgM
MHC protein (class I)
MHC protein (class II)
CD8

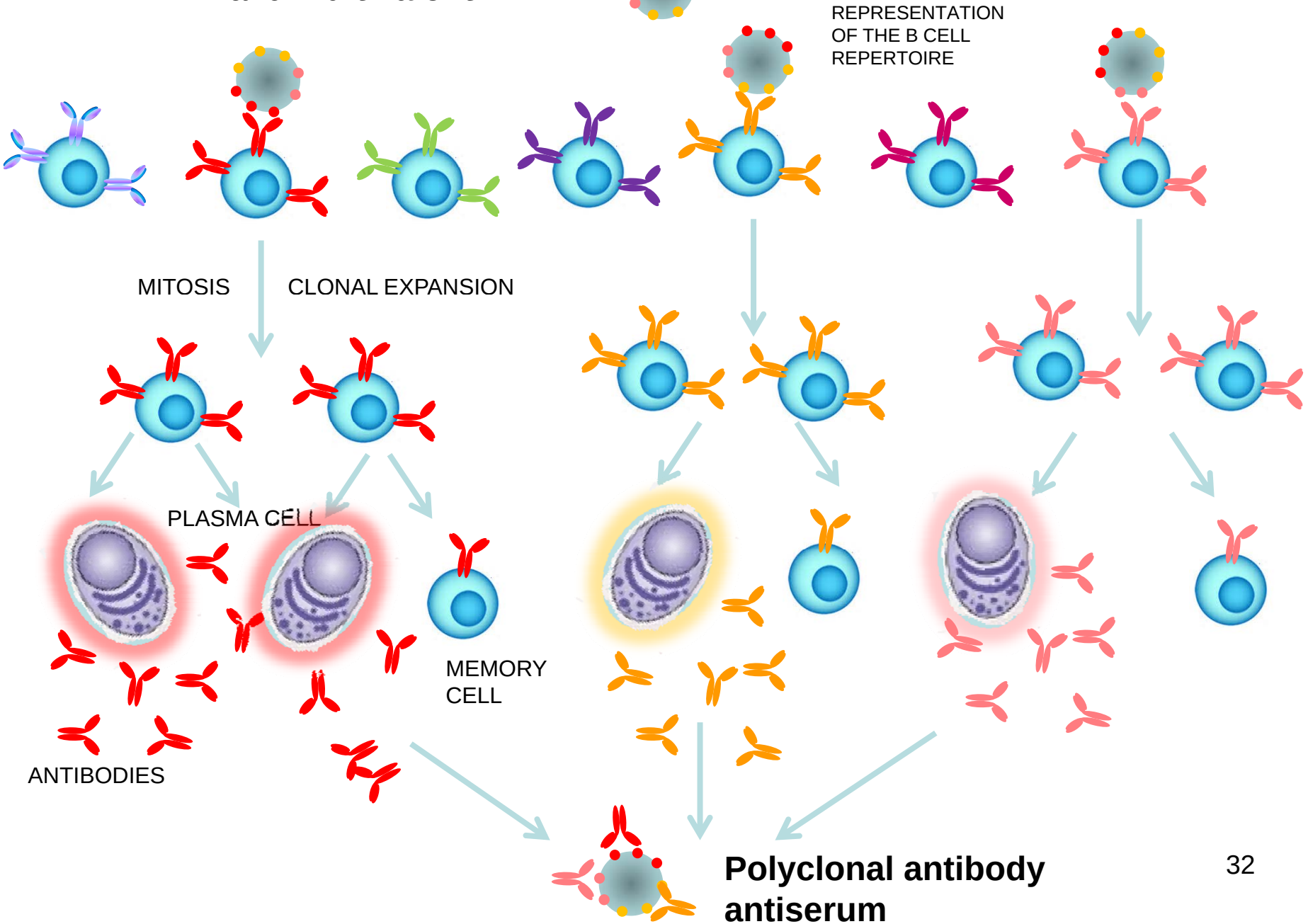
Simplified scheme of the immune response



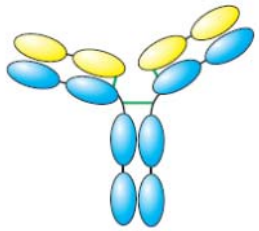
multideterminate
and **multivalent?**

Antigen

The Immune System



Polyclonal antibody antiserum



Antibodies

The conformations of target antigen and binding site are complementary

Antibody specificity and affinity

Antigen–antibody reactions can show a high level of specificity. For example, antibodies to measles virus will bind to the measles virus and confer immunity to this disease, but will not combine with, or protect against, an unrelated virus such as polio. The specificity of an antiserum is equal to the sum of the actions of every antibody in that antiserum. The antibody population may contain many antigen-binding sites, each reacting with a different epitope, or even with different parts of the same epitope (*Fig. 4.16*). However, when some of the epitopes of an antigen, A, are shared by another antigen, B, then a proportion of the antibodies directed to A will also react with B. This phenomenon is termed cross-reactivity.

Specificity, cross-reactivity and non-reactivity

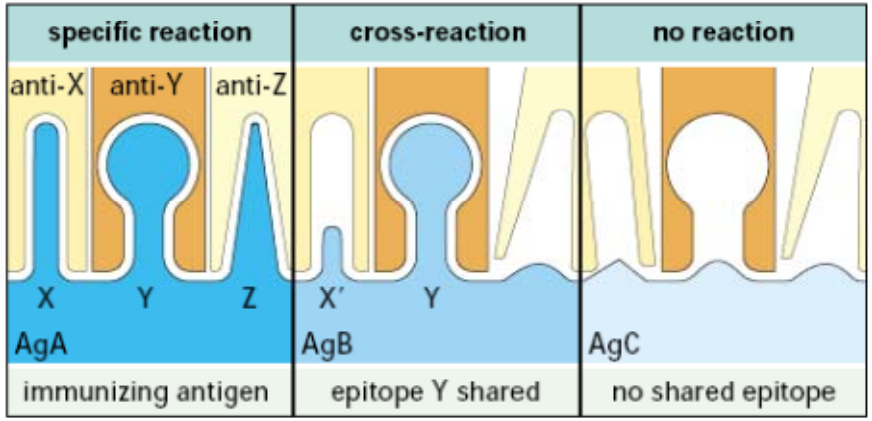
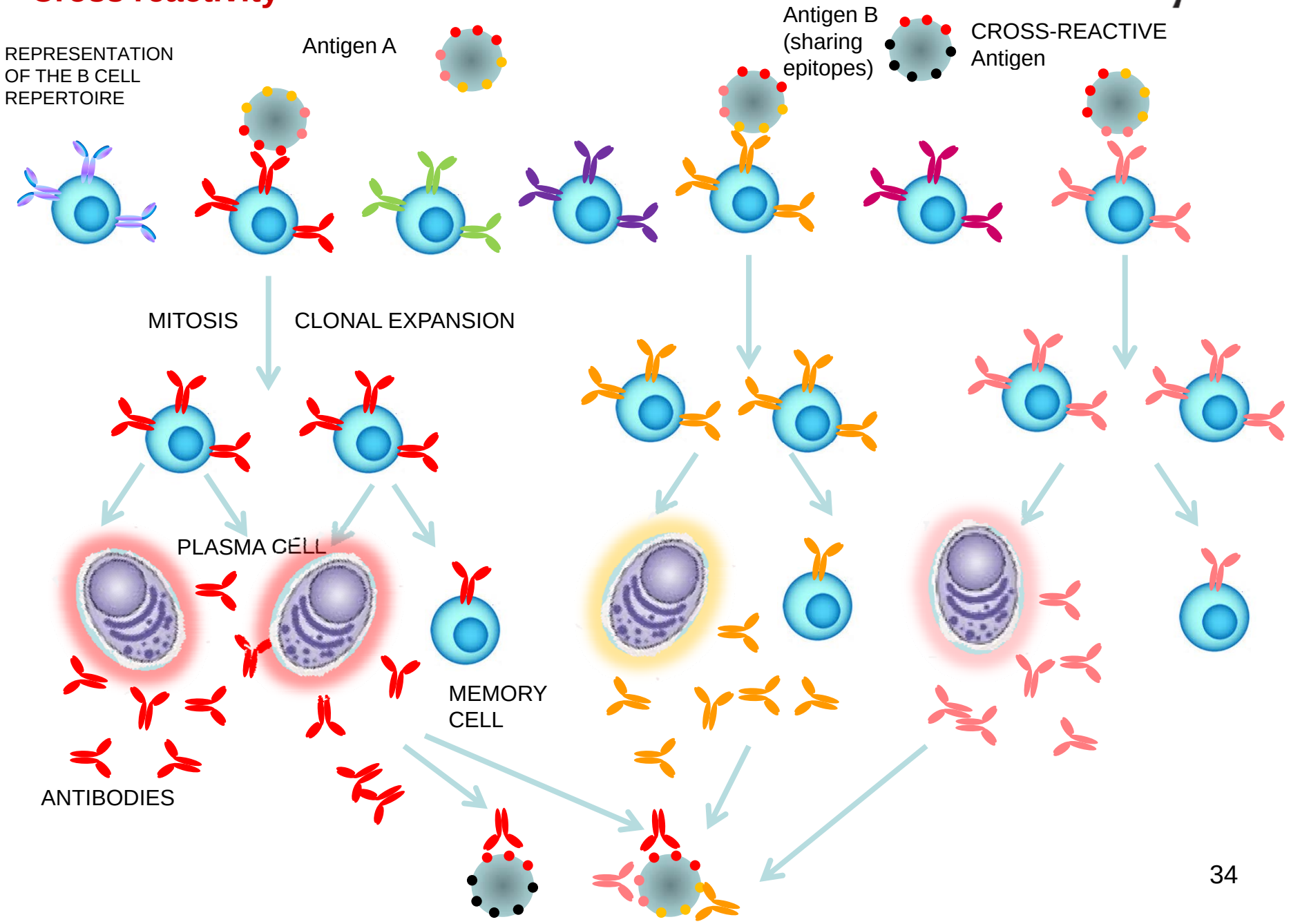
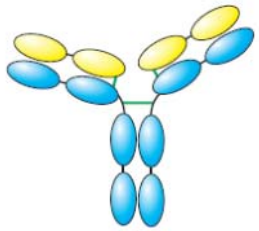


Fig. 4.16 Antiserum specificity results from the action of a population of individual antibody molecules (anti-X, anti-Y, anti-Z) directed against different epitopes (X, Y, Z) on the same or different antigen molecules. Antigen A (AgA) and antigen B (AgB) have epitope Y in common. Antiserum raised against AgA (anti-XYZ) not only reacts specifically with AgA, but cross-reacts with AgB (through recognition of epitopes Y and X′). The antiserum gives no reaction with AgC because there are no shared epitopes.

Cross reactivity





Antibodies

Antibodies recognize the overall conformation of antigens

Clearly, antibodies recognize the overall shape of an epitope rather than particular chemical residues (*Fig. 4.17*). Antibodies are capable of expressing remarkable specificity, and are able to distinguish between small differences in the primary amino acid sequence of protein antigens, in addition to differences in charge, optical configuration and steric conformation. One consequence of this specificity is that many antibodies will bind only to native antigens, or to fragments of antigens that retain sufficient tertiary structure to permit the multiple interactions required for bond formation (*Fig. 4.18*).

When considering antibodies which bind to protein antigens, we can distinguish some which interact with epitopes consisting of a single contiguous stretch of amino acids (a continuous epitope) from others which bind to epitopes formed from separated segments of the polypeptide chain (discontinuous epitopes). Antibodies which bind to discontinuous epitopes often do not bind to denatured antigens e.g. in Western blots.

Specificity and cross-reactivity

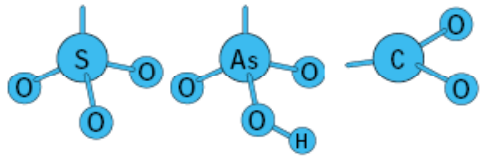
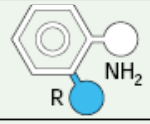
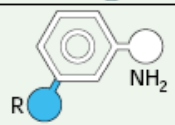
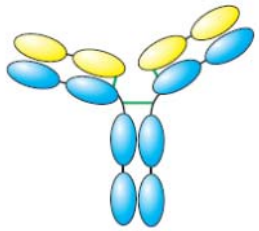
radical (R)	sulphonate	arsonate	carboxylate
			
	tetrahedral	tetrahedral	planar
ortho 	++	−	−
meta 	+++	+	±
para 	±	−	−

Fig. 4.17 Antiserum raised to the meta isomer of aminobenzene sulphonate (the immunizing hapten), is mixed with ortho and para isomers of aminobenzene sulphonate, and also with the three isomers (ortho, meta, para) of two different but related antigens: aminobenzene arsonate and aminobenzene carboxylate. The antiserum reacts specifically with the sulphonate group in the meta position, but will cross-react (although more weakly) with sulphonate in the ortho position. Further, weaker cross-reactions are possible when the antiserum is reacted with either the arsonate group or the carboxylate group in the meta, but not in the ortho or para position. Arsonate is larger than sulphonate and has an extra hydrogen atom, while carboxylate is the smallest. These data suggest that an antigen's configuration is as important as the individual chemical groupings that it contains.



Antibodies

Configurational specificity

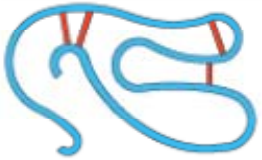
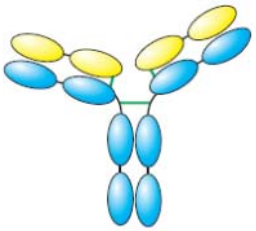
antiserum	antigen		
	lysozyme	isolated 'loop' peptide	reduced 'loop'
			
anti-lysozyme	+ +	+	–
anti-'loop' peptide	+	+ +	–

Fig. 4.18 The lysozyme molecule possesses an intrachain bond (red) which produces a loop in the peptide chain. Antisera raised against whole lysozyme (anti-lysozyme) and the isolated loop (anti-'loop' peptide), are able to distinguish between the two. Neither antiserum reacts with the isolated loop in its linear, reduced form. This demonstrates the importance of tertiary structure in determining antibody specificity.



Antibodies

FUNCTIONS OF ANTIBODIES

The primary function of an antibody is to bind antigen. In a few cases this has a direct effect, for example by neutralizing bacterial toxin, or by preventing viral attachment to host cells. In general, however, the interaction of antibody and antigen is without significance unless secondary 'effector' functions come into play (*Fig. 4.19*).

Selected effector functions of human immunoglobulins

Function		immunoglobulin									
		IgG1	IgG2	IgG3	IgG4	IgM	IgA1	IgA2	sIgA	IgD	IgE
complement fixation (classical pathway)		++	+	+++	–	+++	–	–	–	–	–
placental transfer		+	+	+	+	–	–	–	–	–	–
Binding to cell surface receptors on:											
mononuclear cells	FcγRI	++	–	+++	++	–	–	–	–	–	–
	FcγRIIa	+	(+)	++	–	–	–	–	–	–	–
	FcγRIIIa	+	–	+	–	–	–	–	–	–	–
	FcμR	–	–	–	–	+	–	–	–	–	–
	FcεRII	–	–	–	–	–	–	–	–	–	++
	FcαR	–	–	–	–	–	++	++	++	–	–
neutrophils	FcγRIIa	+	–	+	–	–	–	–	–	–	–
	FcγRIIIb	+	–	+	–	–	–	–	–	–	–
	FcαR	–	–	–	–	–	++	++	++	–	–
mast cells/ basophils	FcεRI	–	–	–	–	–	–	–	–	–	+++

Fig. 4.19 These effector functions are associated with different parts of the Fc region. Placental transfer of IgG in man and intestinal transport in rodents are mediated by an MHC class-I-like receptor molecule (see *Fig. 4.24*). A complex family of receptor molecules able to bind immunoglobulin continues to be discovered (selected examples are listed here). FcμR is expressed by activated B cells but not by T cells or monocytes. FcεRII is also expressed on eosinophils, platelets, T cells and B cells.

FUNCTIONS OF ANTIBODIES

IgG class – IgG is the most important class of immunoglobulin in secondary immune responses and, unlike IgM, is distributed evenly between the intravascular and extravascular pools.

A major effector mechanism of the human IgG1 and IgG3 subclasses is the activation of the classical pathway of complement. The latter is a complex group of serum proteins involved in the elimination of pathogens and the mediation of inflammation (see Chapter 3). The IgG2 subclass is less effective at complement activation and IgG4 appears to be inactive.

In humans IgG molecules of all subclasses cross the placenta and confer a high degree of passive immunity to the newborn (*Fig. 4.20*). In some species, e.g. the pig, maternal immunoglobulin is only transferred to the offspring postnatally. In such cases there is a selective transport of such IgG across the gastrointestinal tract via a specific receptor. The IgG subclasses also interact with a complex array of Fc receptors expressed on various cells, as summarized in *Figure 4.19* and discussed further in the section below.

IgM class – IgM is the predominant antibody in primary immune responses. The protein is largely confined to the intravascular pool and is frequently associated with the immune response to antigenically complex, blood-borne infectious organisms.

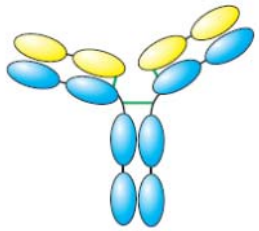
Once bound to its target, IgM is a potent activator of the classical pathway complement. Unlike IgG-mediated activation where two antibody molecules in close apposition are required, a single molecule of bound IgM is able to initiate the cascade because adjacent Fc regions are intrinsic to the structure.

IgA class – Although there are significant levels of IgA in human serum it is generally accepted that the secretory form of the protein is, in a functional sense, the most important. Secretory IgA is assembled during an active transport process as locally produced dimeric IgA passes across mucosal epithelium (*Fig. 4.21*).

In human serum IgA1 is the predominant subclass (approximately 90% of total IgA) and in many secretions such as nasal secretions, tears, saliva and milk IgA1 will account for 70–95% of total IgA. However, in the colon IgA2 predominates (approximately 60% of the total IgA). It is of interest that many microorganisms in the upper respiratory tract have adapted to their environment by releasing proteases that cleave IgA1.

IgD class – The precise biological function of this class of immunoglobulin remains unclear although it may play a role in antigen-triggered lymphocyte differentiation.

IgE class – Despite its low serum concentration, the IgE class is characterized by its ability to bind avidly to circulating basophils and tissue mast cells through the high affinity FcεRI receptor (see next section). It also sensitizes cells on mucosal surfaces such as the conjunctival, nasal and bronchial mucosae. This class of immunoglobulin may have evolved to provide immunity against helminthic parasites but in developed countries it is now more commonly associated with allergic diseases such as asthma and hay fever.



Antibodies

Immunoglobulins in the serum of the fetus and newborn child

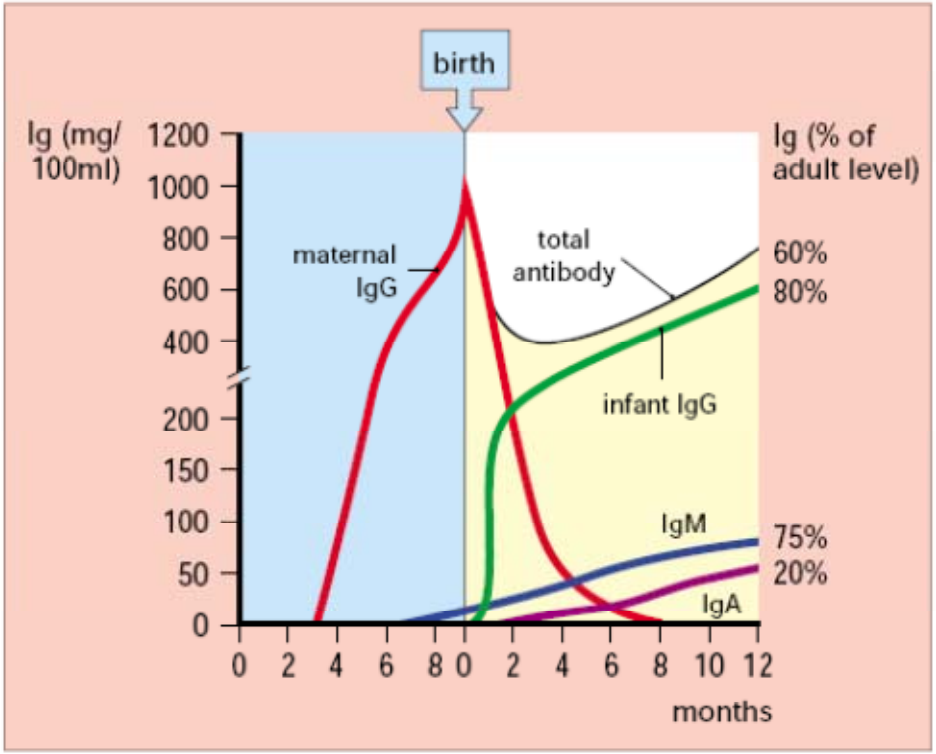
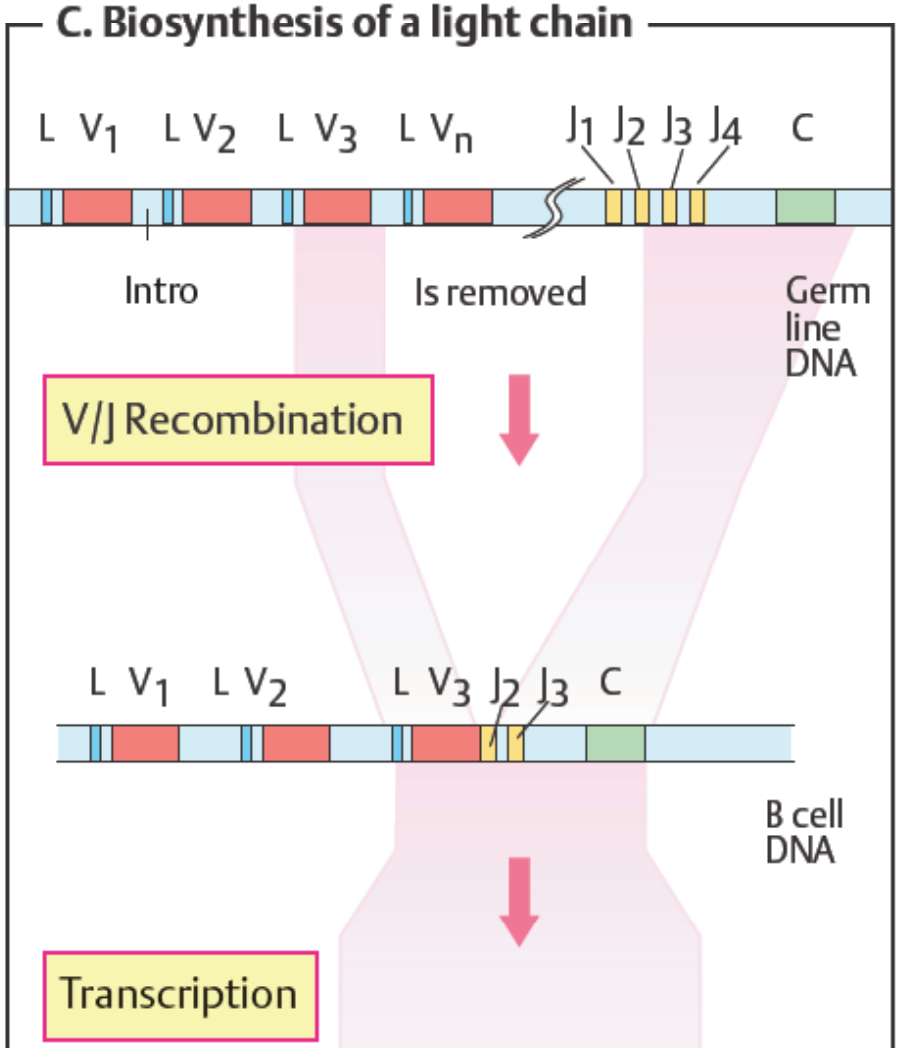


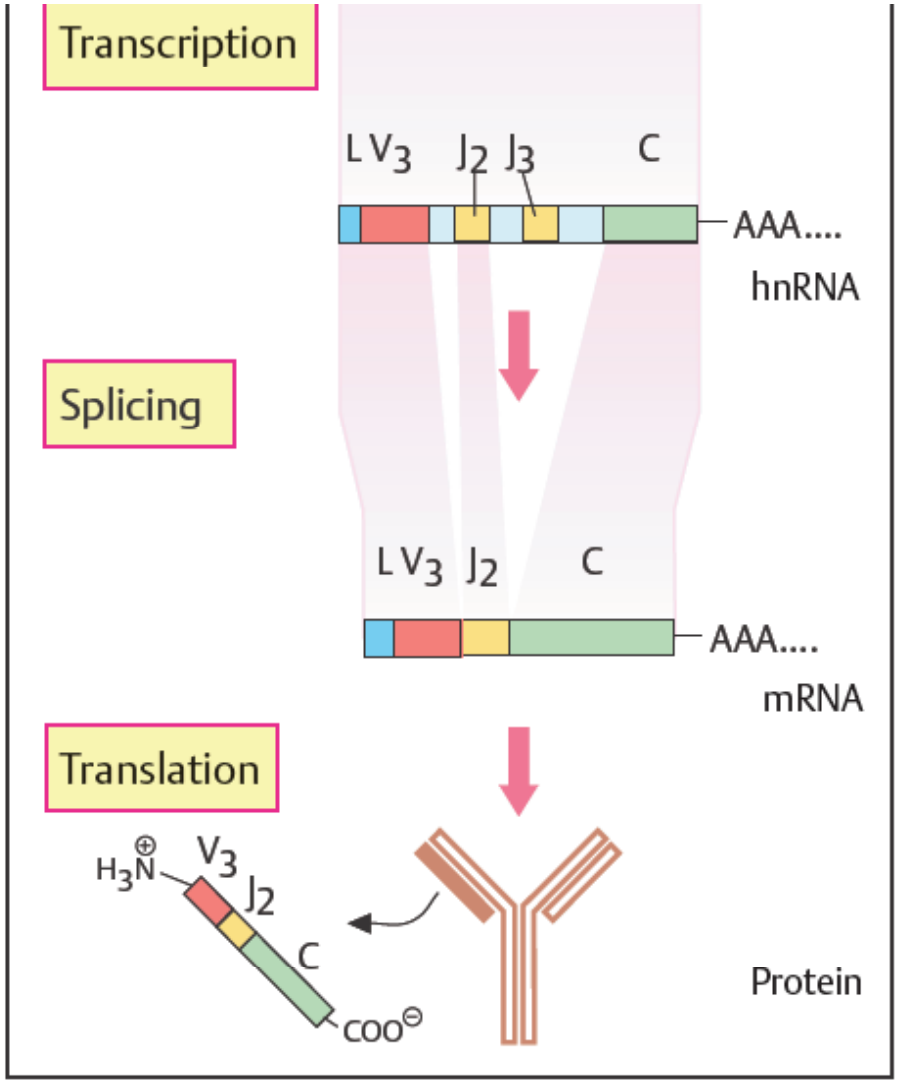
Fig. 4.20 IgG in the fetus and newborn infant is derived solely from the mother. This maternal IgG has disappeared by the age of 9 months, by which time the infant is synthesizing its own IgG. The neonate produces its own IgM and IgA; these classes cannot cross the placenta. By the age of 12 months, the infant produces 80% of its adult level of IgG, 75% of its adult IgM level and 20% of its adult IgA level.



C. Biosynthesis of a light chain ○

We can look at the basic features of the genetic organization and synthesis of immunoglobulins using the biosynthesis of a mouse κ chain as an example. The gene segments for this light chain are designated L, V, J, and C. They are located on chromosome 6 in the **germ-line DNA** (on chromosome 2 in humans) and are separated from one another by introns (see p. 242) of different lengths.

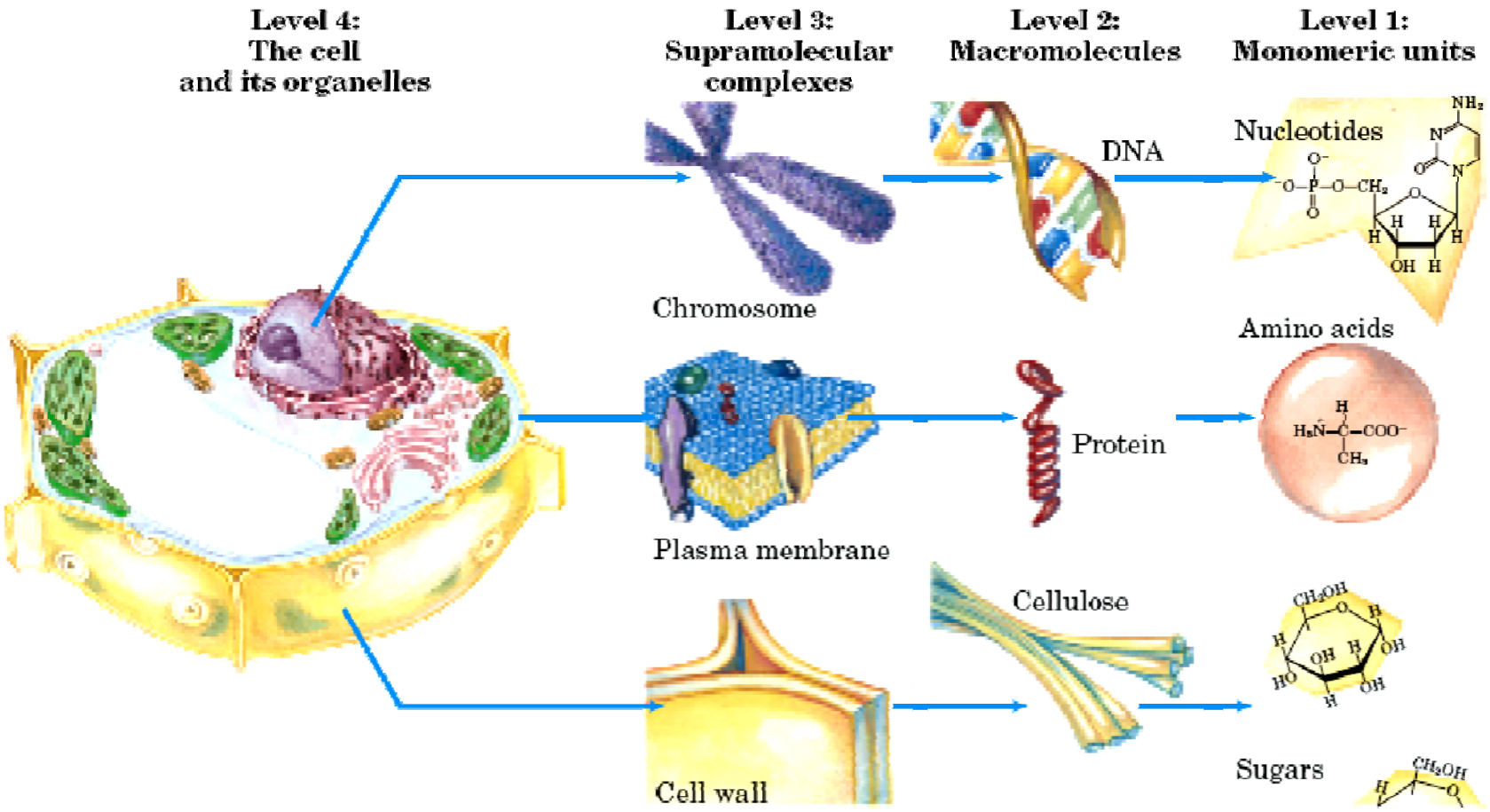
Some 150 identical **L segments** code for the signal peptide ("leader sequence," 17–20 amino acids) for secretion of the product (see p. 230). The **V segments**, of which there are 150 different variants, code for most of the variable domains (95 of the 108 amino acids). L and V segments always occur in pairs—in tandem, so to speak. By contrast, there are only five variants of the **J segments** (joining segments) at most. These code for a peptide with 13 amino acids that links the variable part of the κ chains to the constant part. A single **C segment** codes for the constant part of the light chain (84 amino acids).

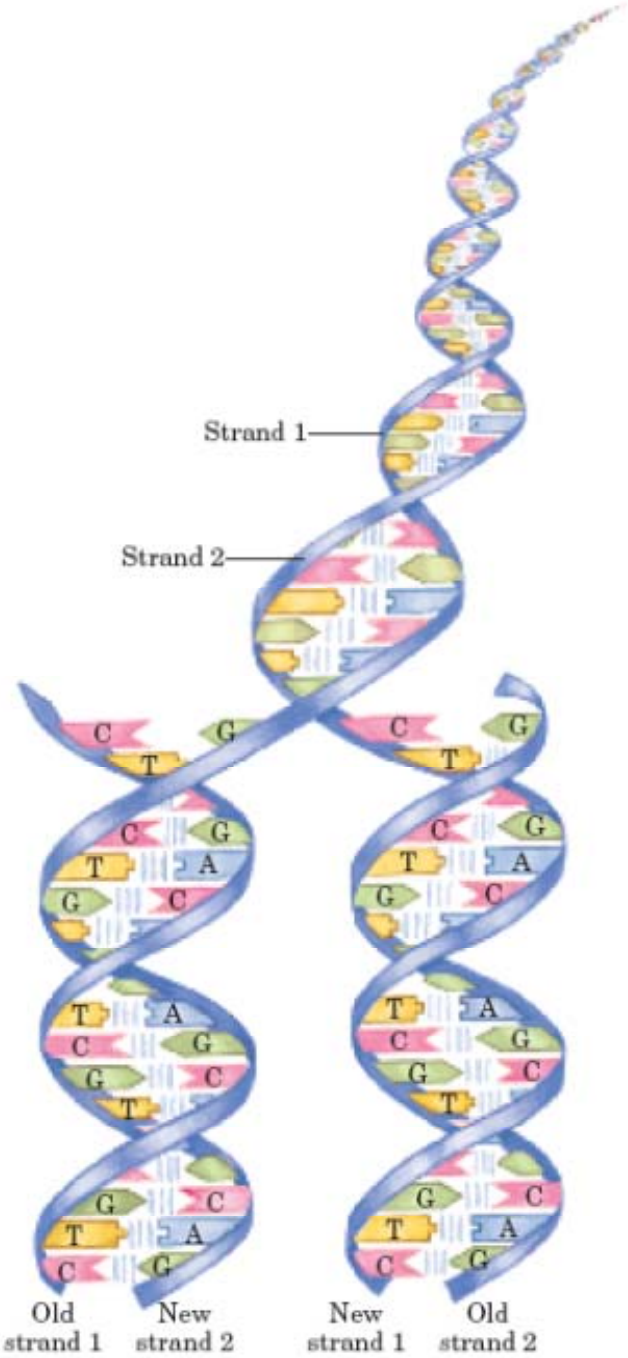


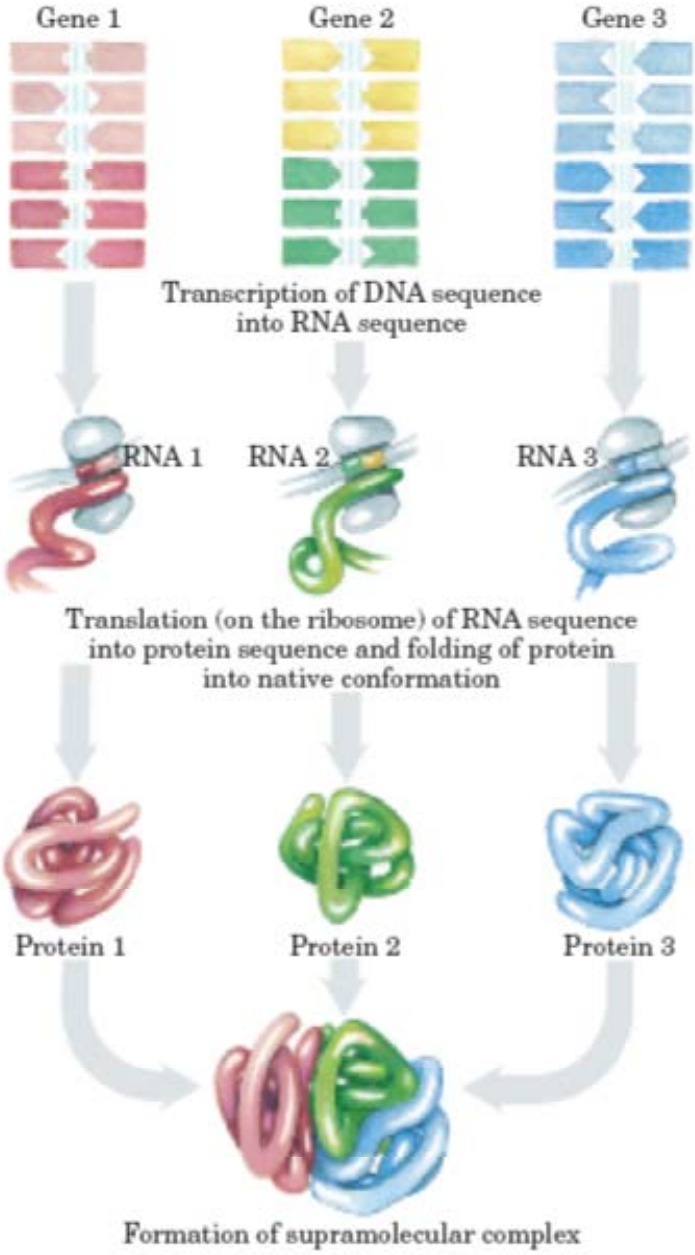
C. Biosynthesis of a light chain ○

(continuación)

During the differentiation of B lymphocytes, individual *V/J combinations* arise in each B cell. One of the 150 L/V tandem segments is selected and linked to one of the five J segments. This gives rise to a *somatic gene* that is much smaller than the germline gene. Transcription of this gene leads to the formation of the **hnRNA** for the κ chain, from which introns and surplus J segments are removed by splicing (see p. 246). Finally, the completed **mRNA** still contains one each of the L–V–J–C segments and after being transported into the cytoplasm is available for translation. The subsequent steps in Ig biosynthesis follow the rules for the synthesis of membrane-bound or secretory proteins (see p. 230).

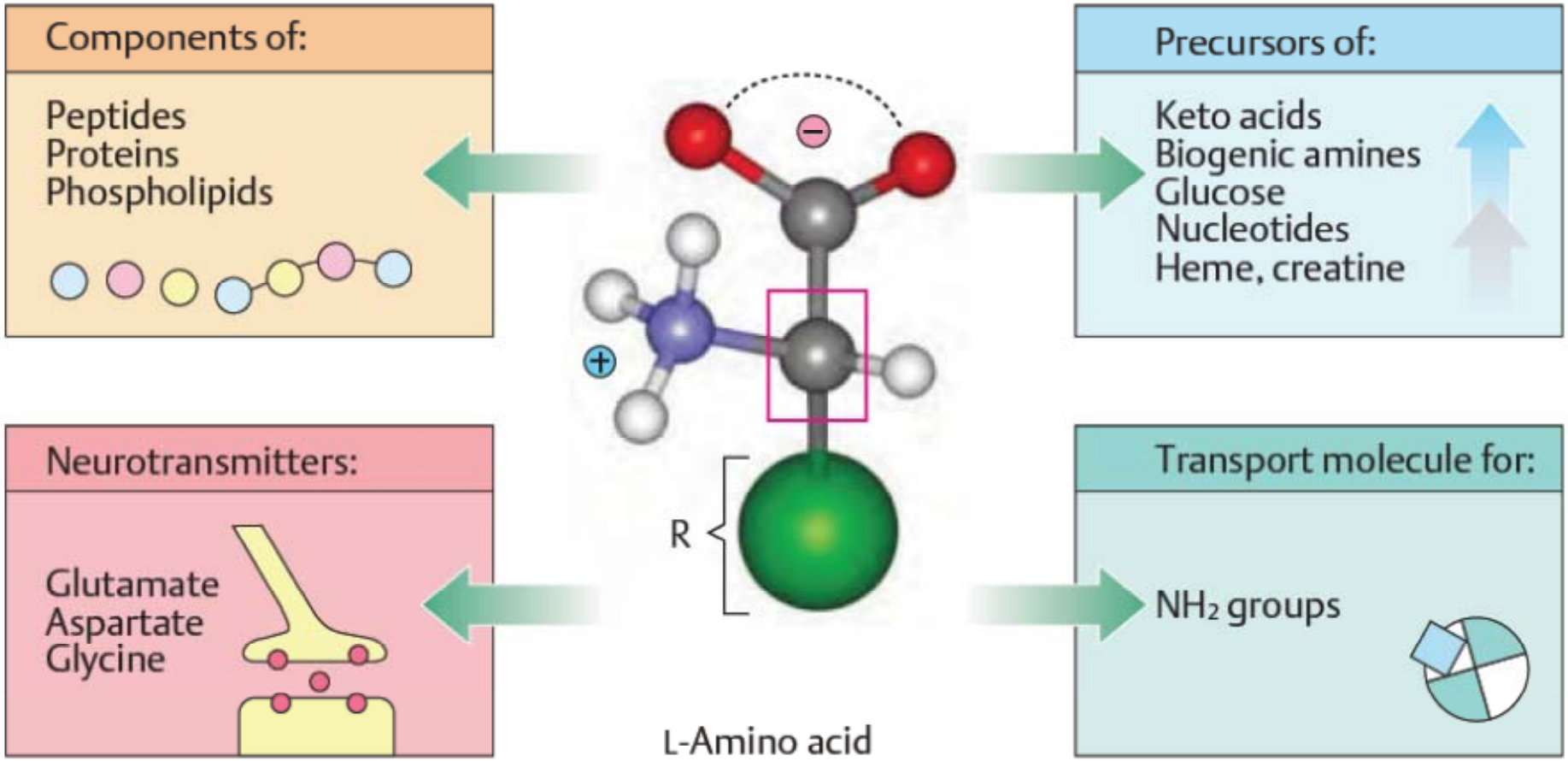

Level 4:
The cell
and its organelles
Level 3:
Supramolecular
complexes
Level 2:
Macromolecules
Level 1:
Monomeric units
Chromosome
DNA
Nucleotides
Amino acids
Plasma membrane
Protein
Cellulose
Cell wall
Sugars


Strand 1
Strand 2
Old strand 1
New strand 2
New strand 1
Old strand 2

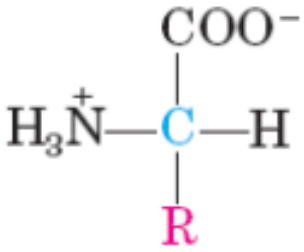

Gene 1
Gene 2
Gene 3
Transcription of DNA sequence into RNA sequence
RNA 1
RNA 2
RNA 3
Translation (on the ribosome) of RNA sequence into protein sequence and folding of protein into native conformation
Protein 1
Protein 2
Protein 3
Formation of supramolecular complex

AMINO ACIDS

A. Amino acids: functions



AMINO ACIDS

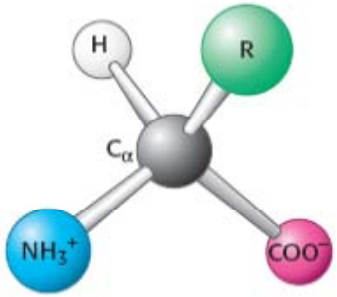


PROPERTIES OF AMINO ACIDS

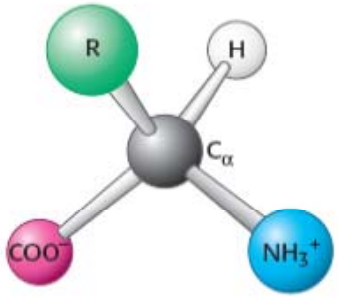
The Genetic Code Specifies 20 L-α-Amino Acids

General structure of an amino acid. This structure is common to all but one of the α-amino acids. (Proline, a cyclic amino acid, is the exception.) The R group or side chain (red) attached to the α carbon (blue) is different in each amino acid.

Of the over 300 naturally occurring amino acids, 20 constitute the monomer units of proteins.



L isomer



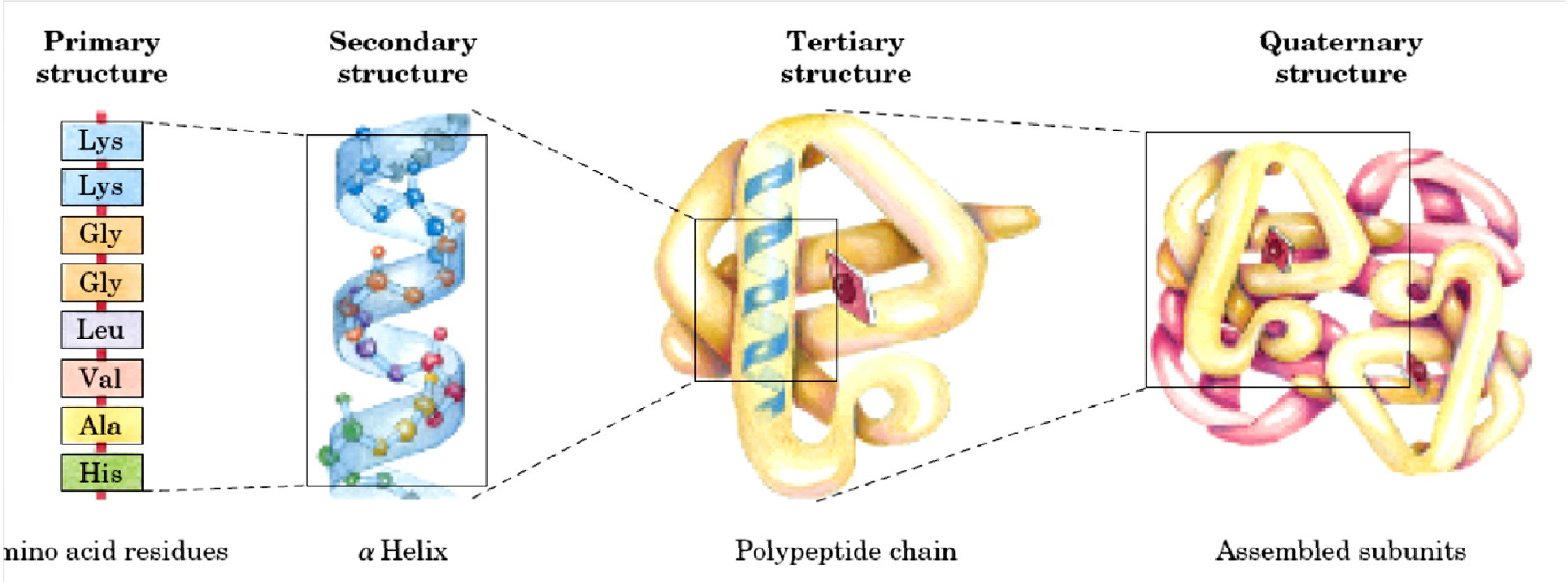
D isomer

The Amino Acid Residues in Proteins Are L Stereoisomers

(b) L-Alanine D-Alanine

PROTEINS

There Are Several Levels of Protein Structure



PROTEINS

THE FOUR ORDERS OF PROTEIN STRUCTURE

The modular nature of protein synthesis and folding are embodied in the concept of orders of protein structure: **primary structure,** the sequence of the amino acids in a polypeptide chain; **secondary structure,** the folding of short (3- to 30-residue), contiguous segments of polypeptide into geometrically ordered units; **tertiary structure,** the three-dimensional assembly of secondary structural units to form larger functional units such as the mature polypeptide and its component domains; and **quaternary structure,** the number and types of polypeptide units of oligomeric proteins and their spatial arrangement.