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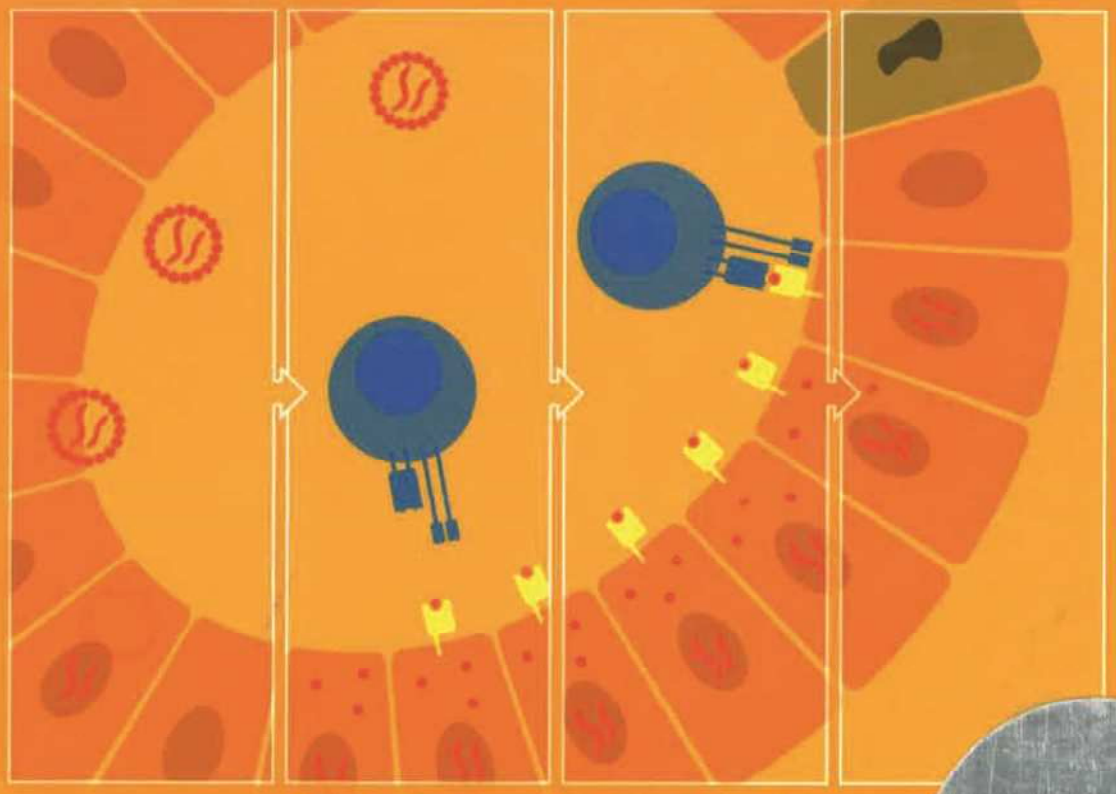
THE IMMUNE SYSTEM IN HEALTH AND DISEASE



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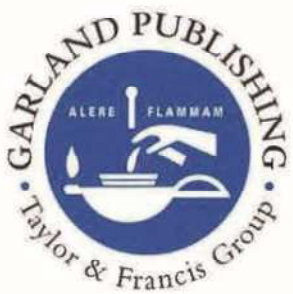

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Antigen Recognition by B-cell and T-cell Receptors

3

We have learned in Chapter 2 that the body is defended by innate immune responses, but these will only work to control pathogens that have certain molecular patterns or that induce interferons and other secreted yet non-specific defenses. Most crucially, they do not allow memory to form as they operate by receptors that are coded in the genome. Thus, innate immunity is good for preventing pathogens from growing freely in the body, but it does not lead to the most important feature of adaptive immunity, which is long-lasting memory of specific pathogen.

To recognize and fight the wide range of pathogens an individual will encounter, the lymphocytes of the adaptive immune system have evolved to recognize a great variety of different antigens from bacteria, viruses, and other disease-causing organisms. The antigen-recognition molecules of B cells are the **immunoglobulins**, or **Ig**. These proteins are produced by B cells in a vast range of antigen specificities, each B cell producing immunoglobulin of a single specificity (see Sections 1-8 to 1-10). Membrane-bound immunoglobulin on the B-cell surface serves as the cell's receptor for antigen, and is known as the **B-cell receptor (BCR)**. Immunoglobulin of the same antigen specificity is secreted as **antibody** by terminally differentiated B cells—the plasma cells. The secretion of antibodies, which bind pathogens or their toxic products in the extracellular spaces of the body, is the main effector function of B cells in adaptive immunity.

Antibodies were the first molecules involved in specific immune recognition to be characterized and are still the best understood. The antibody molecule has two separate functions: one is to bind specifically to molecules from the pathogen that elicited the immune response; the other is to recruit other cells and molecules to destroy the pathogen once the antibody is bound to it. For example, binding by antibody neutralizes viruses and marks pathogens for destruction by phagocytes and complement, as described in Section 1-14. These functions are structurally separated in the antibody molecule, one part of which specifically recognizes and binds to the pathogen or antigen whereas the other engages different effector mechanisms. The antigen-binding region varies extensively between antibody molecules and is thus known as the **variable region** or **V region**. The variability of antibody molecules allows each antibody to bind a different specific antigen, and the total repertoire of antibodies made by a single individual is large enough to ensure that virtually any structure can be recognized. The region of the antibody molecule that engages the effector functions of the immune system does not vary in the same way and is thus known as the **constant region** or **C region**. It comes in five main forms, which are each specialized for activating different effector mechanisms. The membrane-bound B-cell receptor does not have these effector functions, as the C region remains inserted in the membrane of the B cell. Its function is as a receptor that recognizes and binds antigen by the V regions exposed on the surface of the cell, thus transmitting a signal that causes B-cell activation leading to clonal expansion and specific antibody production.

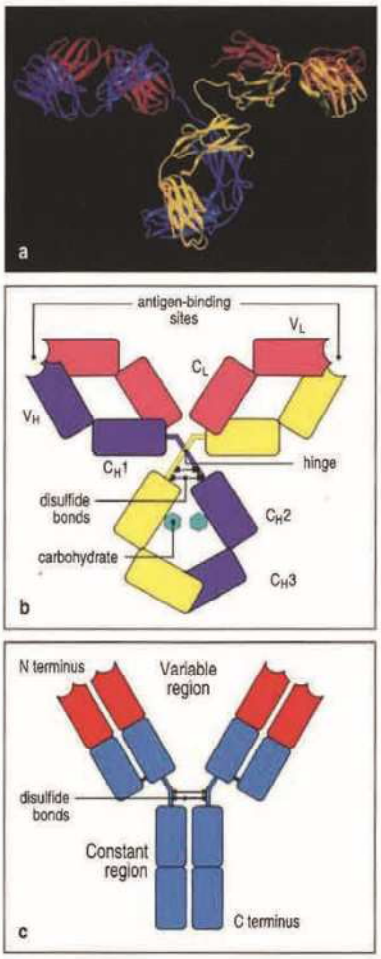


Fig. 3.1 Structure of an antibody molecule. Panel a illustrates a ribbon diagram based on the X-ray crystallographic structure of an IgG antibody, showing the course of the backbones of the polypeptide chains. Three globular regions form a Y. The two antigen-binding sites are at the tips of the arms, which are tethered to the trunk of the Y by a flexible hinge region. A schematic representation of the structure in a is given in panel b, illustrating the four-chain composition and the separate domains comprising each chain. Panel c shows a simplified schematic representation of an antibody molecule that will be used throughout this book. Photograph courtesy of A. McPherson and L. Harris.

The antigen-recognition molecules of T cells are made solely as membrane-bound proteins and only function to signal T cells for activation. These **T-cell receptors** (TCRs) are related to immunoglobulins both in their protein structure—having both V and C regions—and in the genetic mechanism that produces their great variability (see Section 1-10 and Chapter 4). However, the T-cell receptor differs from the B-cell receptor in an important way: it does not recognize and bind antigen directly, but instead recognizes short peptide fragments of pathogen protein antigens, which are bound to **MHC molecules** on the surfaces of other cells.

The MHC molecules are glycoproteins encoded in the large cluster of genes known as the **major histocompatibility complex** (MHC) (see Sections 1-16 and 1-17). Their most striking structural feature is a cleft running across their outermost surface, in which a variety of peptides can be bound. As we shall discuss further in Chapter 5, MHC molecules show great genetic variation in the population, and each individual carries up to 12 of the possible variants, which increases the range of pathogen-derived peptides that can be bound. T-cell receptors recognize features both of the peptide antigen and of the MHC molecule to which it is bound. This introduces an extra dimension to antigen recognition by T cells, known as **MHC restriction**, because any given T-cell receptor is specific not simply for a foreign peptide antigen, but for a unique combination of a peptide and a particular MHC molecule. The ability of T-cell receptors to recognize MHC molecules, and their selection during T-cell development for the ability to recognize the particular MHC molecules expressed by an individual, are topics we shall return to in Chapters 5 and 7.

In this chapter we focus on the structure and antigen-binding properties of immunoglobulins and T-cell receptors. Although B cells and T cells recognize foreign molecules in two distinct fashions, the receptor molecules they use for this task are very similar in structure. We will see how this basic structure can accommodate great variability in antigen specificity, and how it enables immunoglobulins and T-cell receptors to carry out their functions as the antigen-recognition molecules of the adaptive immune response.

The structure of a typical antibody molecule.

Antibodies are the secreted form of the B-cell receptor. An antibody is identical to the B-cell receptor of the cell that secretes it except for a small portion of the C-terminus of the heavy-chain constant region. In the case of the B-cell receptor the C-terminus is a hydrophobic membrane-anchoring sequence, and in the case of antibody it is a hydrophilic sequence that allows secretion. Since they are soluble, and secreted in large quantities, antibodies are easily obtainable and easily studied. For this reason, most of what we know about the B-cell receptor comes from the study of antibodies.

Antibody molecules are roughly Y-shaped molecules consisting of three equal-sized portions, loosely connected by a flexible tether. Three schematic representations of antibody structure, which has been determined by X-ray crystallography, are shown in Fig. 3.1. The aim of this part of the chapter is to explain how this structure is formed and how it allows antibody molecules to carry out their dual tasks—binding on the one hand to a wide variety of antigens, and on the other hand to a limited number of effector molecules and cells. As we will see, each of these tasks is carried out by separable parts of the molecule. The two arms of the Y end in regions that vary between different

antibody molecules, the V regions. These are involved in antigen binding, whereas the stem of the Y, or the C region, is far less variable and is the part that interacts with effector cells and molecules.

All antibodies are constructed in the same way from paired heavy and light polypeptide chains, and the generic term immunoglobulin is used for all such proteins. Within this general category, however, five different **classes** of immunoglobulins—IgM, IgD, IgG, IgA, and IgE—can be distinguished by their C regions, which will be described more fully in Chapter 4. More subtle differences confined to the V region account for the specificity of antigen binding. We will use the IgG antibody molecule as an example to describe the general structural features of immunoglobulins.

3-1 IgG antibodies consist of four polypeptide chains.

IgG antibodies are large molecules, having a molecular weight of approximately 150 kDa, composed of two different kinds of polypeptide chain. One, of approximately 50 kDa, is termed the **heavy** or **H chain**, and the other, of 25 kDa, is termed the **light** or **L chain** (Fig. 3.2). Each IgG molecule consists of two heavy chains and two light chains. The two heavy chains are linked to each other by disulfide bonds and each heavy chain is linked to a light chain by a disulfide bond. In any given immunoglobulin molecule, the two heavy chains and the two light chains are identical, giving an antibody molecule two identical antigen-binding sites (see Fig. 3.1), and thus the ability to bind simultaneously to two identical structures.

Two types of light chain, termed **lambda** (λ) and **kappa** (κ), are found in antibodies. A given immunoglobulin either has κ chains or λ chains, never one of each. No functional difference has been found between antibodies having λ or κ light chains, and either type of light chain may be found in antibodies of any of the five major classes. The ratio of the two types of light chain varies from species to species. In mice, the average κ to λ ratio is 20:1, whereas in humans it is 2:1 and in cattle it is 1:20. The reason for this variation is unknown. Distortions of this ratio can sometimes be used to detect the abnormal proliferation of a clone of B cells. These would all express the identical light chain, and thus an excess of λ light chains in a person might indicate the presence of a B-cell tumor producing λ chains.

By contrast, the class, and thus the effector function, of an antibody, is defined by the structure of its heavy chain. There are five main **heavy-chain classes** or **isotypes**, some of which have several subtypes, and these determine the functional activity of an antibody molecule. The five major classes of immunoglobulin are **immunoglobulin M (IgM)**, **immunoglobulin D (IgD)**, **immunoglobulin G (IgG)**, **immunoglobulin A (IgA)**, and **immunoglobulin E (IgE)**. Their heavy chains are denoted by the corresponding lower-case Greek letter (μ, δ, γ, α, and ε, respectively). IgG is by far the most abundant immunoglobulin and has several subclasses (IgG1, 2, 3, and 4 in humans). Their distinctive functional properties are conferred by the carboxy-terminal part of the heavy chain, where it is not associated with the light chain. We will describe the structure and functions of the different heavy-chain isotypes in Chapter 4. The general structural features of all the isotypes are similar and we will consider IgG, the most abundant isotype in plasma, as a typical antibody molecule.

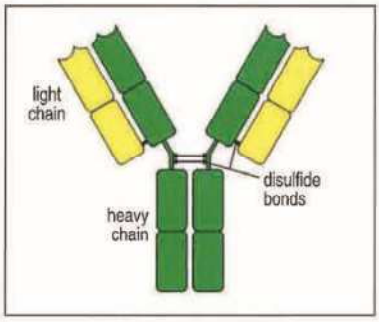


Fig. 3.2 Immunoglobulin molecules are composed of two types of protein chain: heavy chains and light chains. Each immunoglobulin molecule is made up of two heavy chains (green) and two light chains (yellow) joined by disulfide bonds so that each heavy chain is linked to a light chain and the two heavy chains are linked together.

3-2 Immunoglobulin heavy and light chains are composed of constant and variable regions.

The amino acid sequences of many immunoglobulin heavy and light chains have been determined and reveal two important features of antibody molecules. First, each chain consists of a series of similar, although not identical, sequences, each about 110 amino acids long. Each of these repeats corresponds to a discrete, compactly folded region of protein structure known as a protein domain. The light chain is made up of two such **immunoglobulin domains**, whereas the heavy chain of the IgG antibody contains four (see Fig. 3.1a). This suggests that the immunoglobulin chains have evolved by repeated duplication of an ancestral gene corresponding to a single domain.

The second important feature revealed by comparisons of amino acid sequences is that the amino-terminal sequences of both the heavy and light chains vary greatly between different antibodies. The variability in sequence is limited to approximately the first 110 amino acids, corresponding to the first domain, whereas the remaining domains are constant between immunoglobulin chains of the same isotype. The amino-terminal variable or **V domains** of the heavy and light chains (V_H and V_L, respectively) together make up the V region of the antibody and confer on it the ability to bind specific antigen, while the constant domains (**C domains**) of the heavy and light chains (C_H and C_L, respectively) make up the C region (see Fig. 3.1b, c). The multiple heavy-chain C domains are numbered from the amino-terminal end to the carboxy terminus, for example C_H1, C_H2, and so on.

3-3 The antibody molecule can readily be cleaved into functionally distinct fragments.

The protein domains described above associate to form larger globular domains. Thus, when fully folded and assembled, an antibody molecule comprises three equal-sized globular portions joined by a flexible stretch of polypeptide chain known as the **hinge region** (see Fig. 3.1b). Each arm of this Y-shaped structure is formed by the association of a light chain with the amino-terminal half of a heavy chain, whereas the trunk of the Y is formed by the pairing of the carboxy-terminal halves of the two heavy chains. The association of the heavy and light chains is such that the V_H and V_L domains are paired, as are the C_H1 and C_L domains. The C_H3 domains pair with each other but the C_H2 domains do not interact; carbohydrate side chains attached to the C_H2 domains lie between the two heavy chains. The two antigen-binding sites are formed by the paired V_H and V_L domains at the ends of the two arms of the Y (see Fig. 3.1b).

Proteolytic enzymes (proteases) that cleave polypeptide sequences have been used to dissect the structure of antibody molecules and to determine which parts of the molecule are responsible for its various functions. Limited digestion with the protease papain cleaves antibody molecules into three fragments (Fig. 3.3). Two fragments are identical and contain the antigen-binding activity. These are termed the **Fab fragments**, for Fragment antigen binding. The Fab fragments correspond to the two identical arms of the antibody molecule, which contain the complete light chains paired with the V_H and C_H1 domains of the heavy chains. The other fragment contains no antigen-binding activity but was originally observed to crystallize readily, and for this reason was named the **Fc fragment**, for Fragment crystallizable. This fragment corresponds to the paired C_H2 and C_H3 domains and is the part of the antibody molecule that interacts with effector molecules and cells. The functional differences between heavy-chain isotypes lie mainly in the Fc fragment.

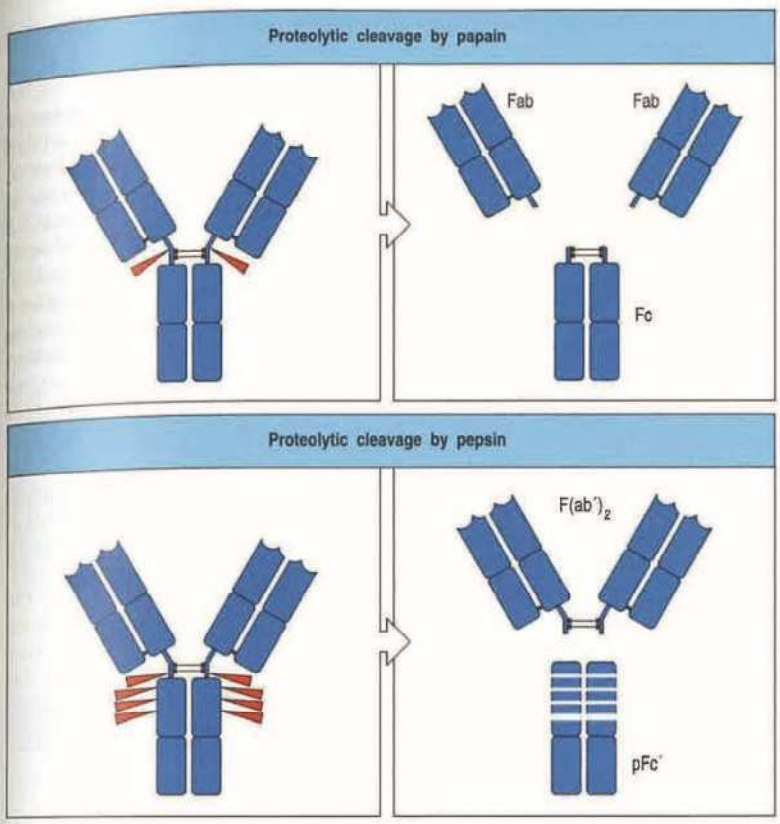


Fig. 3.3 The Y-shaped immunoglobulin molecule can be dissected by partial digestion with proteases. Papain cleaves the immunoglobulin molecule into three pieces, two Fab fragments and one Fc fragment (upper panels). The Fab fragment contains the V regions and binds antigen. The Fc fragment is crystallizable and contains C regions. Pepsin cleaves immunoglobulin to yield one $F(ab')_2$ fragment and many small pieces of the Fc fragment, the largest of which is called the pFc′ fragment (lower panels). $F(ab')_2$ is written with a prime because it contains a few more amino acids than Fab, including the cysteines that form the disulfide bonds.

The protein fragments obtained after proteolysis are determined by where the protease cuts the antibody molecule in relation to the disulfide bonds that link the two heavy chains. These lie in the hinge region between the C_H1 and C_H2 domains and, as illustrated in Fig. 3.3, papain cleaves the antibody molecule on the amino-terminal side of the disulfide bonds. This releases the two arms of the antibody as separate Fab fragments, whereas in the Fc fragment the carboxy-terminal halves of the heavy chains remain linked.

Another protease, pepsin, cuts in the same general region of the antibody molecule as papain but on the carboxy-terminal side of the disulfide bonds (see Fig. 3.3). This produces a fragment, the **$F(ab')_2$ fragment**, in which the two antigen-binding arms of the antibody molecule remain linked. In this case the remaining part of the heavy chain is cut into several small fragments. The $F(ab')_2$ fragment has exactly the same antigen-binding characteristics as the original antibody but is unable to interact with any effector molecule. It is thus of potential value in therapeutic applications of antibodies as well as in research into the functional role of the Fc portion.

Genetic engineering techniques also now permit the construction of many different antibody-related molecules. One important type is a truncated Fab comprising only the V domain of a heavy chain linked by a stretch of synthetic peptide to a V domain of a light chain. This is called **single-chain Fv**, named from Fragment variable. Fv molecules may become valuable therapeutic agents because of their small size, which allows them to penetrate tissues readily. They can be coupled to protein toxins to yield immunotoxins with potential application, for example, in tumor therapy in the case of a Fv specific for a tumor antigen (see Chapter 14).

3-4 The immunoglobulin molecule is flexible, especially at the hinge region.

The hinge region that links the Fc and Fab portions of the antibody molecule is in reality a flexible tether, allowing independent movement of the two Fab arms, rather than a rigid hinge. This has been demonstrated by electron microscopy of antibodies bound to **haptens**. These are small molecules of various sorts, typically about the size of a tyrosine side chain. They can be recognized by antibody but are only able to stimulate production of anti-hapten antibodies when linked to a larger protein **carrier** (see Appendix I, Section A-1). An antigen made of two identical hapten molecules joined by a short flexible region can link two or more anti-hapten antibodies, forming dimers, trimers, tetramers, and so on, which can be seen by electron microscopy (Fig. 3.4). The shapes formed by these complexes demonstrate that antibody molecules are flexible at the hinge region. Some flexibility is also found at the junction between the V and C domains, allowing bending and rotation of the V domain relative to the C domain. For example, in the antibody molecule shown in Fig. 3.1a, not only are the two hinge regions clearly bent differently, but the angle between the V and C domains in each of the two Fab arms is also different. This range of motion has led to the junction between the V and C domains being referred to as a 'molecular ball-and-socket joint.' Flexibility at both the hinge and V–C junction enables the binding of both arms of an antibody molecule to sites that are various distances apart, for example, sites on bacterial cell-wall polysaccharides. Flexibility at the hinge also enables the antibodies to interact with the antibody-binding proteins that mediate immune effector mechanisms.

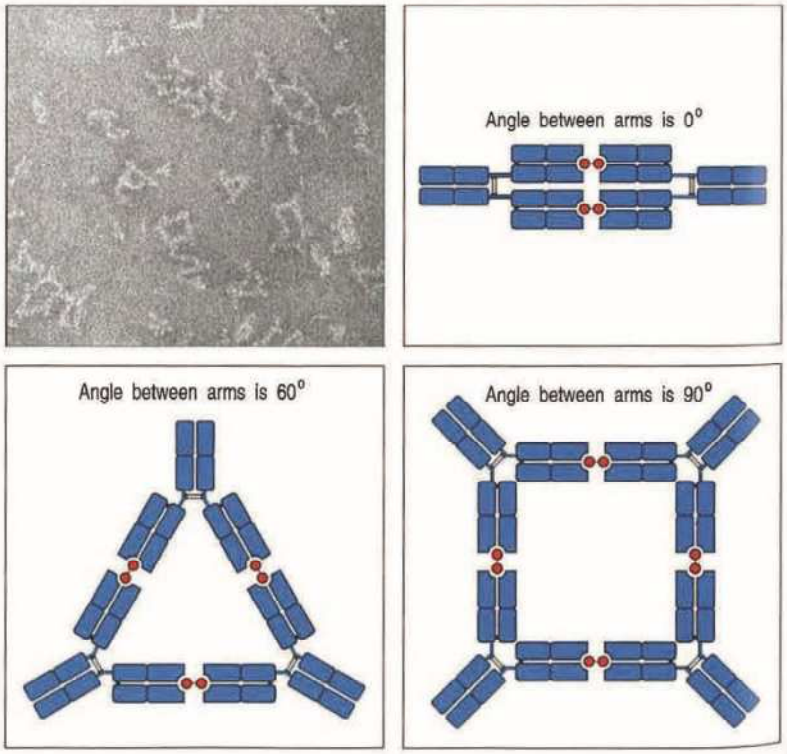


Fig. 3.4 Antibody arms are joined by a flexible hinge. An antigen consisting of two hapten molecules (red balls in diagrams) that can cross-link two antigen-binding sites is used to create antigen:antibody complexes, which can be seen in the electron micrograph. Linear, triangular, and square forms are seen, with short projections or spikes. Limited pepsin digestion removes these spikes (not shown in the figure), which therefore correspond to the Fc portion of the antibody; the $F(ab')_2$ pieces remain cross-linked by antigen. The interpretation of the complexes is shown in the diagrams. The angle between the arms of the antibody molecules varies, from 0° in the antibody dimers, through 60° in the triangular forms, to 90° in the square forms, showing that the connections between the arms are flexible. Photograph (× 300,000) courtesy of N.M. Green.

3-5 The domains of an immunoglobulin molecule have similar structures.

As we saw in Section 3-2, immunoglobulin heavy and light chains are composed of a series of discrete protein domains. These protein domains all have a similar folded structure. Within this basic three-dimensional structure, there are distinct differences between V and C domains. The structural similarities and differences can be seen in the diagram of a light chain in Fig. 3.5. Each domain is constructed from two **β sheets**, which are elements of protein structure made up of strands of the polypeptide chain (β strands) packed together; the sheets are linked by a disulfide bridge and together form a roughly barrel-shaped structure, known as a **β barrel**. The distinctive folded structure of the immunoglobulin protein domain is known as the **immunoglobulin fold**.

Both the essential similarity of V and C domains and the critical difference between them are most clearly seen in the bottom panels of Fig. 3.5, where the cylindrical domains are opened out to reveal how the polypeptide chain folds to create each of the β sheets and how it forms flexible loops as it changes direction. The main difference between the V and C domains is that the V domain is larger, with an extra loop. We will see in Section 3-6 that the flexible loops of the V domains form the antigen-binding site of the immunoglobulin molecule.

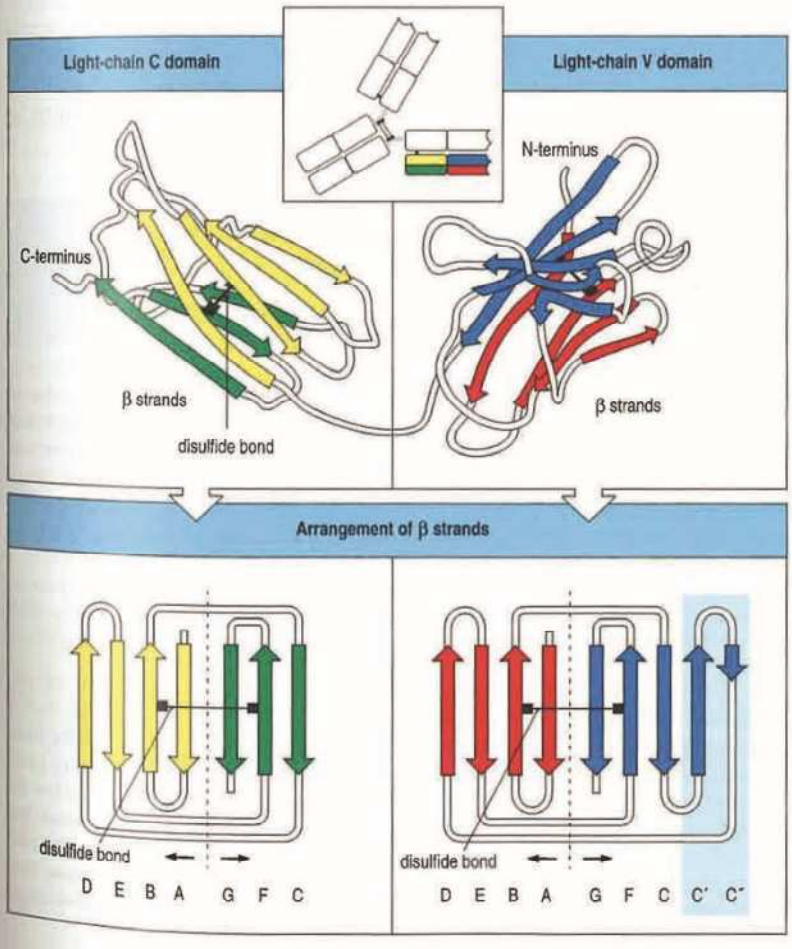


Fig. 3.5 The structure of immunoglobulin constant and variable domains. The upper panels show schematically the folding pattern of the constant (C) and variable (V) domains of an immunoglobulin light chain. Each domain is a barrel-shaped structure in which strands of polypeptide chain (β strands) running in opposite directions (antiparallel) pack together to form two β sheets (shown in yellow and green in the diagram of the C domain), which are held together by a disulfide bond. The way the polypeptide chain folds to give the final structure can be seen more clearly when the sheets are opened out, as shown in the lower panels. The β strands are lettered sequentially with respect to the order of their occurrence in the amino acid sequence of the domains; the order in each β sheet is characteristic of immunoglobulin domains. The β strands C′ and C″ that are found in the V domains but not in the C domains are indicated by a blue shaded background. The characteristic four-strand plus three-strand (C-region type domain) or four-strand plus five-strand (V-region type domain) arrangements are typical immunoglobulin superfamily domain building blocks, found in a whole range of other proteins as well as antibodies and T-cell receptors.

Many of the amino acids that are common to C and V domains of immunoglobulin chains lie in the core of the immunoglobulin fold and are critical to its stability. For that reason, other proteins having sequences similar to those of immunoglobulins are believed to form domains of similar structure, and in many cases this has been demonstrated by crystallography. These **immunoglobulin-like domains** are present in many other proteins of the immune system, and in proteins involved in cell–cell recognition in the nervous system and other tissues. Together with the immunoglobulins and the T-cell receptors, they make up the extensive **immunoglobulin superfamily**.

Summary.

The IgG antibody molecule is made up of four polypeptide chains, comprising two identical light chains and two identical heavy chains, and can be thought of as forming a flexible Y-shaped structure. Each of the four chains has a variable (V) region at its amino terminus, which contributes to the antigen-binding site, and a constant (C) region, which determines the isotype. The isotype of the heavy chain determines the functional properties of the antibody. The light chains are bound to the heavy chains by many non-covalent interactions and by disulfide bonds, and the V regions of the heavy and light chains pair in each arm of the Y to generate two identical antigen-binding sites, which lie at the tips of the arms of the Y. The possession of two antigen-binding sites allows antibody molecules to cross-link antigens and to bind them much more stably. The trunk of the Y, or Fc fragment, is composed of the carboxy-terminal domains of the heavy chains. Joining the arms of the Y to the trunk are the flexible hinge regions. The Fc fragment and hinge regions differ in antibodies of different isotypes, thus determining their functional properties. However, the overall organization of the domains is similar in all isotypes.

The interaction of the antibody molecule with specific antigen.

We have described the structure of the antibody molecule and how the V regions of the heavy and light chains fold and pair to form the antigen-binding site. In this part of the chapter we will look at the antigen-binding site in more detail. We will discuss the different ways in which antigens can bind to antibody and address the question of how variation in the sequences of the antibody V domains determines the specificity for antigen.

3-6 Localized regions of hypervariable sequence form the antigen-binding site.

The V regions of any given antibody molecule differ from those of every other. Sequence variability is not, however, distributed evenly throughout the V regions but is concentrated in certain segments of the V region. The distribution of variable amino acids can be seen clearly in what is termed a **variability plot** (Fig. 3.6), in which the amino acid sequences of many different antibody V regions are compared. Three segments of particular variability can be identified in both the V_H and V_L domains. They are designated **hypervariable regions** and are denoted HV1, HV2, and HV3. In the light chains these are roughly from residues 28 to 35, from 49 to 59, and from 92 to 103, respectively.

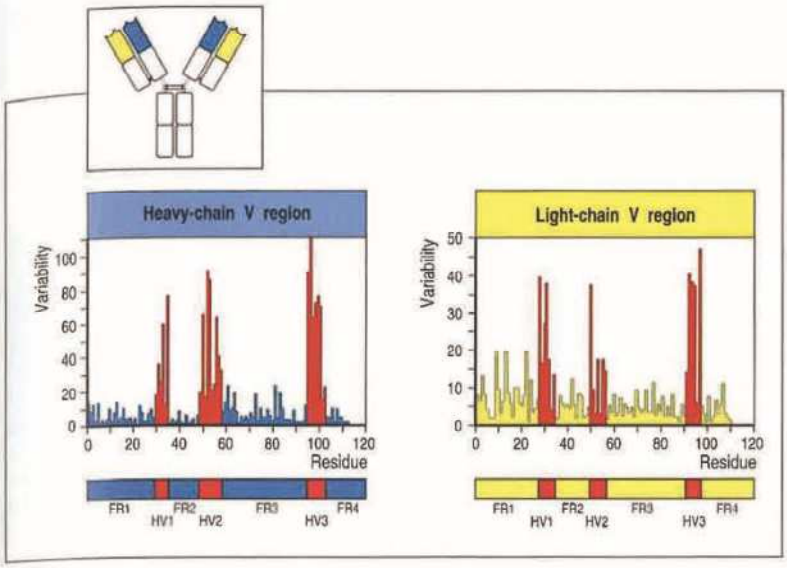


Fig. 3.6 There are discrete regions of hypervariability in V domains. A variability plot derived from comparison of the amino acid sequences of several dozen heavy-chain and light-chain V domains. At each amino acid position the degree of variability is the ratio of the number of different amino acids seen in all of the sequences together to the frequency of the most common amino acid. Three hypervariable regions (HV1, HV2, and HV3) are indicated in red and are also known as the complementarity-determining regions, CDR1, CDR2, and CDR3. They are flanked by less variable framework regions (FR1, FR2, FR3, and FR4, shown in blue or yellow).

The most variable part of the domain is in the HV3 region. The regions between the hypervariable regions, which comprise the rest of the V domain, show less variability and are termed the **framework regions**. There are four such regions in each V domain, designated FR1, FR2, FR3, and FR4.

The framework regions form the β sheets that provide the structural framework of the domain, whereas the hypervariable sequences correspond to three loops at the outer edge of the β barrel, which are juxtaposed in the folded domain (Fig. 3.7). Thus, not only is sequence diversity concentrated in particular parts of the V domain but it is localized to a particular region on the surface of the molecule. When the V_H and V_L domains are paired in the antibody molecule, the hypervariable loops from each domain are brought together, creating a single hypervariable site at the tip of each arm of the molecule. This is the binding site for antigen, the **antigen-binding site** or **antibody combining site**. The three hypervariable loops determine antigen specificity by forming a surface complementary to the antigen, and are more commonly termed the **complementarity-determining regions**, or **CDRs** (**CDR1**, **CDR2**, and **CDR3**). Because CDRs from both V_H and V_L domains contribute to the antigen-binding site, it is the combination of the heavy and the light chain, and not either alone, that determines the final antigen specificity. Thus, one way in which the immune system is able to generate antibodies of different specificities is by generating different combinations of heavy- and light-chain V regions. This means of producing variability is known as **combinatorial diversity**; we will encounter a second form of combinatorial diversity when we consider in Chapter 4 how the genes encoding the heavy- and light-chain V regions are created from smaller segments of DNA.

3-7 Antibodies bind antigens via contacts with amino acids in CDRs, but the details of binding depend upon the size and shape of the antigen.

In early investigations of antigen binding to antibodies, the only available sources of large quantities of a single type of antibody molecule were tumors of antibody-secreting cells. The antigen specificities of the tumor-derived antibodies were unknown, so many compounds had to be screened to identify ligands that could be used to study antigen binding. In general, the substances

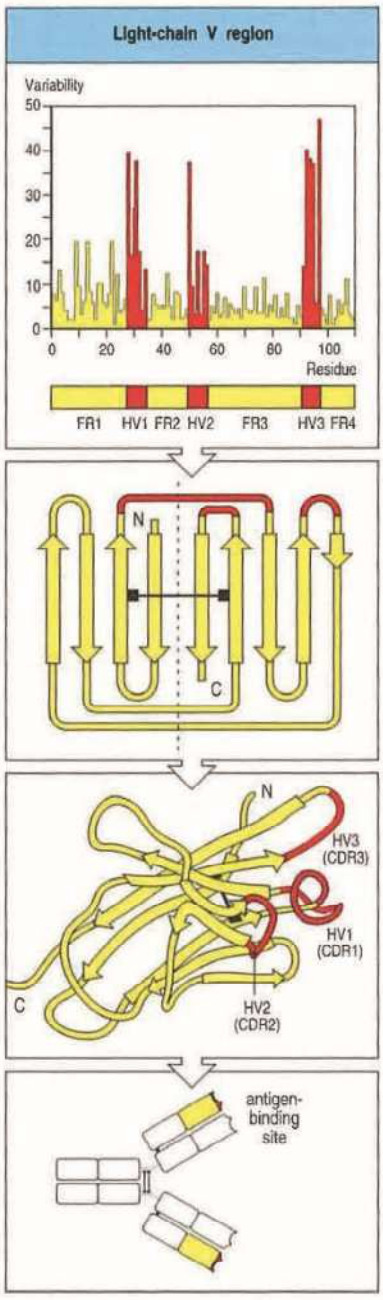


Fig. 3.7 The hypervariable regions lie in discrete loops of the folded structure. When the hypervariable regions (CDRs) are positioned on the structure of a V domain it can be seen that they lie in loops that are brought together in the folded structure. In the antibody molecule, the pairing of a heavy and a light chain brings together the hypervariable loops from each chain to create a single hypervariable surface, which forms the antigen-binding site at the tip of each arm.

found to bind to these antibodies were haptens (see Section 3-4) such as phosphorylcholine or vitamin K_1. Structural analysis of complexes of antibodies with their hapten ligands provided the first direct evidence that the hypervariable regions form the antigen-binding site, and demonstrated the structural basis of specificity for the hapten. Subsequently, with the discovery of methods of generating monoclonal antibodies (see Appendix I, Section A-12), it became possible to make large amounts of pure antibodies specific for many different antigens. This has provided a more general picture of how antibodies interact with their antigens, confirming and extending the view of antibody–antigen interactions derived from the study of haptens.

The surface of the antibody molecule formed by the juxtaposition of the CDRs of the heavy and light chains creates the site to which an antigen binds. Clearly, as the amino acid sequences of the CDRs are different in different antibodies, so are the shapes of the surfaces created by these CDRs. As a general principle, antibodies bind ligands whose surfaces are complementary to that of the antibody. A small antigen, such as a hapten or a short peptide, generally binds in a pocket or groove lying between the heavy- and light-chain V domains (Fig. 3.8, left and center panels). Other antigens, such as a protein molecule, can be of the same size as, or larger than, the antibody molecule itself, and cannot fit into a groove or pocket. In these cases, the interface between the two molecules is often an extended surface that involves all of the CDRs and, in some cases, part of the framework region of the antibody (Fig. 3.8, right panel). This surface need not be concave but can be flat, undulating, or even convex.

3-8 Antibodies bind to conformational shapes on the surfaces of antigens.

The biological function of antibodies is to bind to pathogens and their products, and to facilitate their removal from the body. An antibody generally recognizes only a small region on the surface of a large molecule such as a polysaccharide or protein. The structure recognized by an antibody is called an **antigenic determinant** or **epitope**. Some of the most important pathogens have polysaccharide coats, and antibodies that recognize epitopes formed by the sugar subunits of these molecules are essential in providing immune protection from such pathogens. In many cases, however, the antigens that provoke an immune response are proteins. For example, protective antibodies against viruses recognize viral coat proteins. In such cases, the structures recognized by the antibody are located on the surface of the protein. Such sites are likely to be composed of amino acids from different parts of the polypeptide chain that have been brought together by protein folding. Antigenic determinants of this kind are known as **conformational** or **discontinuous epitopes** because the structure recognized is composed of segments of the protein that are discontinuous in the amino acid sequence of the antigen but are brought together in the three-dimensional structure. In contrast, an epitope composed of a single segment of polypeptide chain is termed a **continuous** or **linear epitope**. Although most antibodies raised

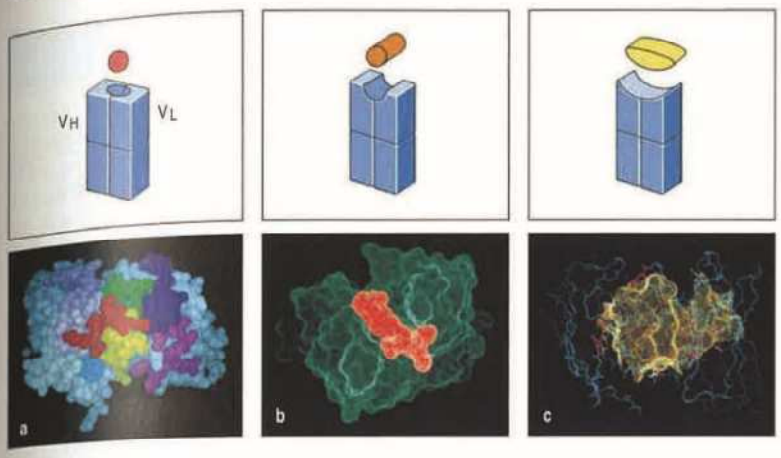


Fig. 3.8 Antigens can bind in pockets or grooves, or on extended surfaces in the binding sites of antibodies. The panels in the top row show schematic representations of the different types of binding site in a Fab fragment of an antibody: left, pocket; center, groove; right, extended surface. Below are examples of each type. Panel a: space-filling representation of the interaction of a small peptide antigen with the complementarity-determining regions (CDRs) of a Fab fragment as viewed looking into the antigen-binding site. Seven amino acid residues of the antigen, shown in red, are bound in the antigen-binding pocket. Five of the six CDRs (H1, H2, H3, L1, and L3) interact with the peptide, whereas L2 does not. The CDR loops are colored as follows: L2, magenta; L3, green; H1, blue; H2, pale purple; H3, yellow. Panel b: in a complex of an antibody with a peptide from the human immunodeficiency virus, the peptide (orange) binds along a groove formed between the heavy- and light-chain V domains (green). Panel c: complex between hen egg-white lysozyme and the Fab fragment of its corresponding antibody (HyHel5). Two extended surfaces come into contact, as can be seen from this computer-generated image, where the surface contour of the lysozyme molecule (yellow dots) is superimposed on the antigen-binding site. Residues in the antibody that make contact with the lysozyme are shown in full (red); for the rest of the Fab fragment only the peptide backbone is shown (blue). All six CDRs of the antibody are involved in the binding. Photographs a and b courtesy of I.A. Wilson and R.L. Stanfield. Photograph c courtesy of S. Sheriff.

against intact, fully folded proteins recognize discontinuous epitopes, some will bind peptide fragments of the protein. Conversely, antibodies raised against peptides of a protein or against synthetic peptides corresponding to part of its sequence are occasionally found to bind to the natural folded protein. This makes it possible, in some cases, to use synthetic peptides in vaccines that aim at raising antibodies against a pathogen protein.

3-9 Antigen–antibody interactions involve a variety of forces.

The interaction between an antibody and its antigen can be disrupted by high salt concentrations, extremes of pH, detergents, and sometimes by competition with high concentrations of the pure epitope itself. The binding is therefore a reversible noncovalent interaction. The forces, or bonds, involved in these noncovalent interactions are outlined in Fig. 3.9.

Electrostatic interactions occur between charged amino acid side chains, as in salt bridges. Interactions also occur between electric dipoles, as in hydrogen bonds, or can involve short-range van der Waals forces. High salt concentrations and extremes of pH disrupt antigen–antibody binding by weakening electrostatic interactions and/or hydrogen bonds. This principle is employed in the purification of antigens using affinity columns of immobilized antibodies, and vice versa for antibody purification (see Appendix I, Section A-5). Hydrophobic interactions occur when two hydrophobic surfaces come together to exclude water. The strength of a hydrophobic interaction is proportional to the surface area that is hidden from water. For some antigens, hydrophobic interactions probably account for most of the binding energy. In some cases, water molecules are trapped in pockets in the interface between antigen and antibody. These trapped water molecules may also contribute to binding, especially between polar amino acid residues.

The contribution of each of these forces to the overall interaction depends on the particular antibody and antigen involved. A striking difference between antibody interactions with protein antigens and most other natural protein–protein interactions is that antibodies possess many aromatic amino acids in their antigen-binding sites. These amino acids participate mainly in van der Waals and hydrophobic interactions, and sometimes in hydrogen bonds. In general, the hydrophobic and van der Waals forces operate over very

Noncovalent forces	Origin	
Electrostatic forces	Attraction between opposite charges	$-\overset{\oplus}{N}H_3 \quad \overset{\ominus}{O}OC-$
Hydrogen bonds	Hydrogen shared between electronegative atoms (N,O)	$>N^{\delta-}-H^{\delta+}\cdots O^{\delta-}=C<$
Van der Waals forces	Fluctuations in electron clouds around molecules oppositely polarize neighboring atoms	$\delta^+\ \delta^- \rightleftharpoons \delta^-\ \delta^+$
Hydrophobic forces	Hydrophobic groups interact unfavorably with water and tend to pack together to exclude water molecules. The attraction also involves van der Waals forces	

Fig. 3.9 The noncovalent forces that hold together the antigen:antibody complex. Partial charges found in electric dipoles are shown as δ^+ or δ^-. Electrostatic forces diminish as the inverse square of the distance separating the charges, whereas van der Waals forces, which are more numerous in most antigen–antibody contacts, fall off as the sixth power of the separation and therefore operate only over very short ranges. Covalent bonds never occur between antigens and naturally produced antibodies.

short ranges and serve to pull together two surfaces that are complementary in shape: hills on one surface must fit into valleys on the other for good binding to occur. In contrast, electrostatic interactions between charged side chains, and hydrogen bonds bridging oxygen and/or nitrogen atoms, accommodate specific features or reactive groups while strengthening the interaction overall.

For example, in the complex of hen egg-white lysozyme with the antibody D1.3 (Fig. 3.10), strong hydrogen bonds are formed between the antibody and a particular glutamine in the lysozyme molecule that protrudes between the V_H and V_L domains. Lysozymes from partridge and turkey have another amino acid in place of the glutamine and do not bind to the antibody. In the high-affinity complex of hen egg-white lysozyme with another antibody, HyHel5 (see Fig. 3.8c), two salt bridges between two basic arginines on the surface of the lysozyme interact with two glutamic acids, one each from the V_H CDR1 and CDR2 loops. Again, lysozymes that lack one of the two arginine residues show a 1000-fold decrease in affinity. Although overall surface complementarity must play an important part in antigen–antibody interactions, specific electrostatic and hydrogen-bonding interactions appear to determine antibody affinity. In most antibodies that have been studied at this level of detail, only a few residues make a major contribution to the binding energy. Genetic engineering by site-directed mutagenesis can further tailor an antibody's binding to its complementary epitope.

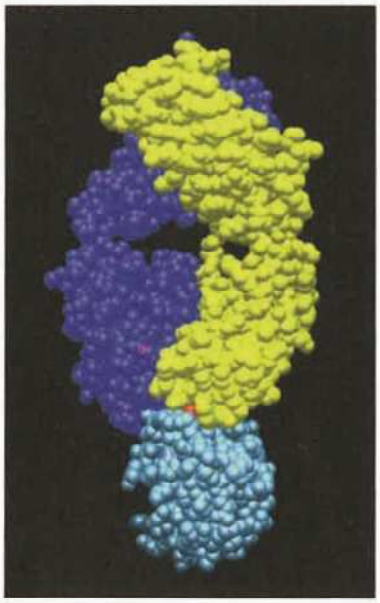


Fig. 3.10 The complex of lysozyme with the antibody D1.3. The interaction of the Fab fragment of D1.3 with hen egg-white lysozyme is shown, with the lysozyme in blue, the heavy chain in purple and the light chain in yellow. A glutamine residue of lysozyme, shown in red, protrudes between the two V domains of the antigen-binding site and makes hydrogen bonds important to the antigen–antibody binding. Original photograph courtesy of R.J. Poljak.

Summary.

X-ray crystallographic analysis of antigen:antibody complexes has demonstrated that the hypervariable loops (complementarity-determining regions) of immunoglobulin V regions determine the specificity of antibodies. With protein antigens, the antibody molecule contacts the antigen over a broad area of its surface that is complementary to the surface recognized on the antigen. Electrostatic interactions, hydrogen bonds, van der Waals forces, and hydrophobic interactions can all contribute to binding. Amino acid side chains in most or all of the hypervariable loops make contact with antigen and determine both the specificity and the affinity of the interaction. Other parts of the V region play little part in the direct contact with the antigen but provide a stable structural framework for the hypervariable loops and help determine their position and conformation. Antibodies raised against intact proteins usually bind to the surface of the protein and make contact with residues that are discontinuous in the primary structure of the molecule; they may, however, occasionally bind peptide fragments of the protein, and antibodies raised against peptides derived from a protein can sometimes be used to detect the native protein molecule. Peptides binding to antibodies usually bind in the cleft between the V regions of the heavy and light chains, where they make specific contact with some, but not necessarily all, of the hypervariable loops. This is also the usual mode of binding for carbohydrate antigens and small molecules such as haptens.

Antigen recognition by T cells.

In contrast to the immunoglobulins, which interact with pathogens and their toxic products in the extracellular spaces of the body, T cells only recognize foreign antigens that are displayed on the surfaces of the body's own cells. These antigens can derive from pathogens such as viruses or intracellular bacteria, which replicate within cells, or from pathogens or their products that cells have internalized by endocytosis from the extracellular fluid.

T cells can detect the presence of an intracellular pathogen because infected cells display on their surface peptide fragments derived from the pathogen's proteins. These foreign peptides are delivered to the cell surface by specialized host-cell glycoproteins. These are encoded in a large cluster of genes that were first identified by their powerful effects on the immune response to transplanted tissues. For that reason, the gene complex was called the **major histocompatibility complex (MHC)**, and the peptide-binding glycoproteins are still known as MHC molecules. The recognition of antigen as a small peptide fragment bound to an MHC molecule and displayed at the cell surface is one of the most distinctive features of T cells, and will be the focus of this part of the chapter. How peptide fragments of antigen become complexed with MHC molecules will be considered in Chapter 5.

In this part of the chapter we will describe the structure and properties of the T-cell antigen receptor, T-cell receptor, or TCR for short. As might be expected from their function as highly variable antigen-recognition structures, T-cell receptors are closely related to antibody molecules in the structure of their genes. There are, however, important differences between T-cell receptors and immunoglobulins that reflect the special features of antigen recognition by the T-cell receptor, and its lack of effector functions.

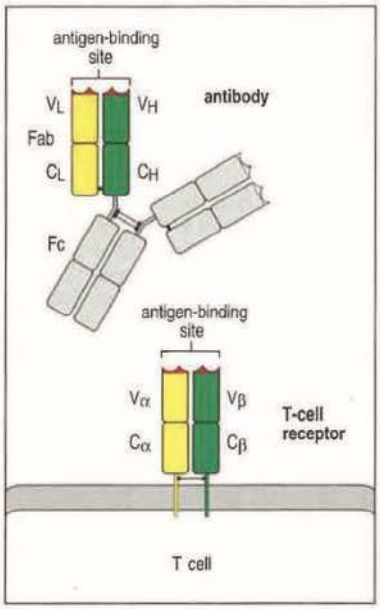


Fig. 3.11 The T-cell receptor resembles a membrane-bound Fab fragment. The Fab fragment of antibody molecules is a disulfide-linked heterodimer, each chain of which contains one immunoglobulin C domain and one V domain; the juxtaposition of the V domains forms the antigen-binding site (see Section 3-6). The T-cell receptor is also a disulfide-linked heterodimer, with each chain containing an immunoglobulin C-like domain and an immunoglobulin V-like domain. As in the Fab fragment, the juxtaposition of the V domains forms the site for antigen recognition.

3-10 The antigen receptor on T cells is very similar to a Fab fragment of immunoglobulin.

T-cell receptors were first identified using monoclonal antibodies that bound only one cloned T-cell line but not others and that could specifically inhibit antigen recognition by that clone of T cells, or specifically activate them (see Appendix I, Section A-19). These **clonotypic** antibodies were then used to show that each T cell bears about 30,000 antigen-receptor molecules on its surface, each receptor consisting of two different polypeptide chains, termed the **T-cell receptor α (TCRα) and β (TCRβ) chains**, linked by a disulfide bond. These α:β heterodimers are very similar in structure to the Fab fragment of an immunoglobulin molecule (Fig. 3.11), and they account for antigen recognition by most T cells. A minority of T cells bear an alternative, but structurally similar, receptor made up of a different pair of polypeptide chains designated γ and δ. **γ:δ T-cell receptors** appear to have different antigen-recognition properties from the **α:β T-cell receptors**, and the function of γ:δ T cells in immune responses is not yet entirely clear. In the rest of this chapter, we shall use the term T-cell receptor to mean the α:β receptor, except where specified otherwise. Both types of T-cell receptor differ from the membrane-bound immunoglobulin that serves as the B-cell receptor: a T-cell receptor has only one antigen-binding site, whereas a B-cell receptor has two, and T-cell receptors are never secreted, whereas immunoglobulin can be secreted as antibody.

Our initial insights into the structure and function of the α:β T-cell receptor came from studies of cloned cDNA encoding the receptor chains. The amino acid sequences predicted from T-cell receptor cDNAs show clearly that both chains of the T-cell receptor have an amino-terminal variable (V) region with homology to an immunoglobulin V domain, a constant (C) region with homology to an immunoglobulin C domain, and a short hinge region containing a cysteine residue that forms the interchain disulfide bond (Fig. 3.12). Each chain spans the lipid bilayer by a hydrophobic transmembrane domain, and ends in a short cytoplasmic tail. These close similarities of T-cell receptor chains to the heavy and light immunoglobulin chains first enabled prediction of the structural resemblance of the T-cell receptor heterodimer to a Fab fragment of immunoglobulin.

Recently, the three-dimensional structure of the T-cell receptor has been determined. The structure is indeed similar to that of an antibody Fab fragment, as was suspected from earlier studies on the genes that encoded it. The T-cell receptor chains fold in much the same way as those of a Fab fragment (Fig. 3.13a), although the final structure appears a little shorter and wider. There are, however, some distinct differences between T-cell receptors and Fab fragments. The most striking difference is in the C_α domain, where the fold is unlike that of any other immunoglobulin-like domain. The half of the domain that is juxtaposed with the C_β domain forms a β sheet similar to that

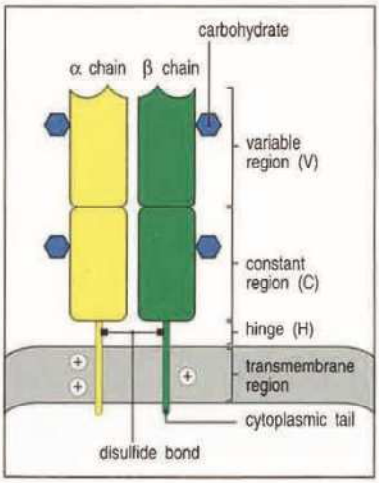


Fig. 3.12 Structure of the T-cell receptor. The T-cell receptor heterodimer is composed of two transmembrane glycoprotein chains, α and β. The extracellular portion of each chain consists of two domains, resembling immunoglobulin V and C domains, respectively. Both chains have carbohydrate side chains attached to each domain. A short segment, analogous to an immunoglobulin hinge region, connects the immunoglobulin-like domains to the membrane and contains the cysteine residue that forms the interchain disulfide bond. The transmembrane helices of both chains are unusual in containing positively charged (basic) residues within the hydrophobic transmembrane segment. The α chains carry two such residues; the β chains have one.

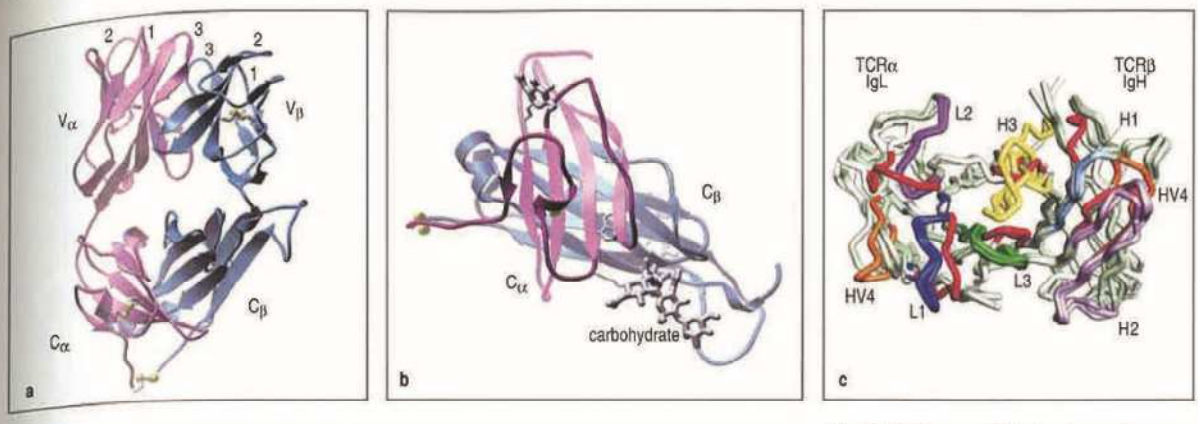


Fig. 3.13 The crystal structure of an α:β T-cell receptor resolved at 2.5 Å. In panels a and b the α chain is shown in pink and the β chain in blue. Disulfide bonds are shown in green. In panel a, the T-cell receptor is viewed from the side as it would sit on a cell surface, with the CDR loops that form the antigen-binding site (labeled 1, 2, and 3) arrayed across its relatively flat top. In panel b, the C_α and C_β domains are shown. The C_α domain does not fold into a typical immunoglobulin-like domain; the face of the domain away from the C_β domain is mainly composed of irregular strands of polypeptide rather than β sheet. The intramolecular disulfide bond joins a β strand to this segment of α helix. The interaction between the C_α and C_β domains is assisted by carbohydrate (colored grey and labeled on the figure), with a sugar group from the C_α domain making hydrogen bonds to the C_β domain. In panel c, the T-cell receptor is shown aligned with the antigen-binding sites from three different antibodies. This view is looking down into the binding site. The V_α domain of the T-cell receptor is aligned with the V_L domains of the antigen-binding sites of the antibodies, and the V_β domain is aligned with the V_H domains. The CDRs of the T-cell receptor and immunoglobulin molecules are colored, with CDRs 1, 2, and 3 of the TCR shown in red and the HV4 loop in orange. For the immunoglobulin V domains, the CDR1 loops of the heavy chain (H1) and light chain (L1) are shown in light and dark blue, respectively, and the CDR2 loops (H2, L2) in light and dark purple, respectively. The heavy-chain CDR3 loops (H3) are in yellow; the light-chain CDR3s (L3) are in bright green. The HV4 loops of the TCR (orange) have no hypervariable counterparts in immunoglobulins. Photographs courtesy of I.A. Wilson.

found in other immunoglobulin-like domains, but the other half of the domain is formed of loosely packed strands and a short segment of α helix (Fig. 3.13b). The intramolecular disulfide bond, which in immunoglobulin-like domains normally joins two β strands, in a C_α domain joins a β strand to this segment of α helix.

There are also differences in the way in which the domains interact. The interface between the V and C domains of both T-cell receptor chains is more extensive than in antibodies, which may make the hinge joint between the domains less flexible. And the interaction between the C_α and C_β domains is distinctive in being assisted by carbohydrate, with a sugar group from the C_α domain making a number of hydrogen bonds to the C_β domain (see Fig. 3.13b). Finally, a comparison of the variable binding sites shows that, although the complementarity-determining region (CDR) loops align fairly closely with those of antibody molecules, there is some displacement relative to those of the antibody molecule (Fig. 3.13c). This displacement is particularly marked in the V_α CDR2 loop, which is oriented at roughly right angles to the equivalent loop in antibody V domains, as a result of a shift in the β strand that anchors one end of the loop from one face of the domain to the other. A strand displacement also causes a change in the orientation of the V_β CDR2 loop in two of the seven V_β domains whose structures are known. As yet, the crystallographic structures of only seven T-cell receptors have been solved to this level of resolution, so it remains to be seen to what degree all T-cell receptors share these features, and whether there is more variability to be discovered.

3-11 A T-cell receptor recognizes antigen in the form of a complex of a foreign peptide bound to an MHC molecule.

Antigen recognition by T-cell receptors clearly differs from recognition by B-cell receptors and antibodies. Antigen recognition by B cells involves direct binding of immunoglobulin to the intact antigen and, as discussed in Section 3-8, antibodies typically bind to the surface of protein antigens, contacting amino acids that are discontinuous in the primary structure but are brought together in the folded protein. T cells, on the other hand, were found to respond to short contiguous amino acid sequences in proteins. These sequences were often buried within the native structure of the protein and thus could not be recognized directly by T-cell receptors unless some unfolding of the protein antigen and its 'processing' into peptide fragments had occurred (Fig. 3.14).

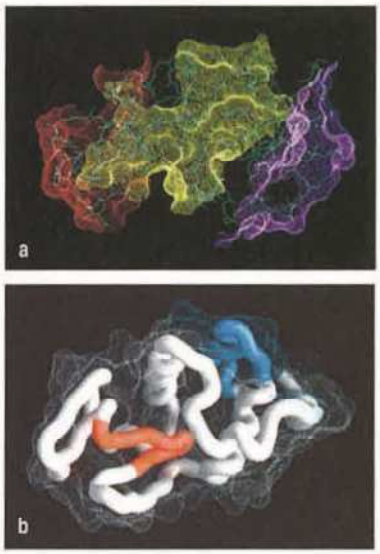


Fig. 3.14 Differences in the recognition of hen egg-white lysozyme by immunoglobulins and T-cell receptors. Antibodies can be shown by X-ray crystallography to bind epitopes on the surface of proteins, as shown in panel a, where the epitopes for three antibodies are shown on the surface of hen egg lysozyme (see also Fig. 3.10). In contrast, the epitopes recognized by T-cell receptors need not lie on the surface of the molecule, as the T-cell receptor recognizes not the antigenic protein itself but a peptide fragment of the protein. The peptides corresponding to two T-cell epitopes of lysozyme are shown in panel b, one epitope, shown in blue, lies on the surface of the protein but a second, shown in red, lies mostly within the core and is inaccessible in the folded protein. For this residue to be accessible to the T-cell receptor, the protein must be unfolded and processed. Panel a courtesy of S. Sheriff.

The nature of the antigen recognized by T cells became clear with the realization that the peptides that stimulate T cells are recognized only when bound to an MHC molecule. These cell-surface glycoproteins are encoded by genes within the major histocompatibility complex (MHC). The ligand recognized by the T cell is thus a complex of peptide and MHC molecule. The evidence for involvement of the MHC in T-cell recognition of antigen was at first indirect, but it has recently been proved conclusively by stimulating T cells with purified peptide:MHC complexes. The T-cell receptor interacts with this ligand by making contacts with both the MHC molecule and the antigen peptide.

3-12 T cells with different functions are distinguished by CD4 and CD8 cell-surface proteins and recognize peptides bound to different classes of MHC molecule.

T cells fall into two major classes that have different effector functions. The two classes are distinguished by the expression of the cell-surface proteins **CD4** and **CD8**. These two types of T cell differ in the class of MHC molecule they recognize. There are two classes of MHC molecule—**MHC class I** and **MHC class II**—which differ in their structure and expression pattern on

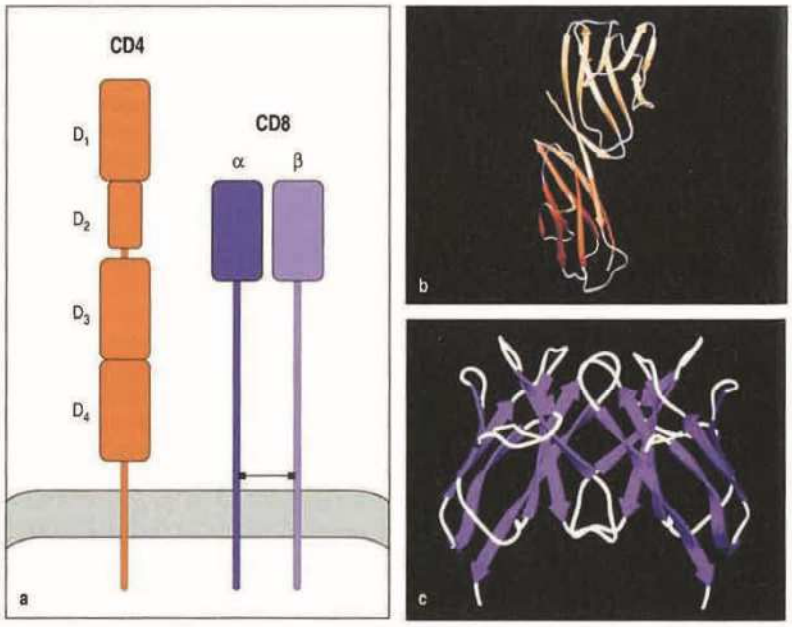


Fig. 3.15 The outline structures of the CD4 and CD8 co-receptor molecules. The CD4 molecule contains four immunoglobulin-like domains, as shown in diagrammatic form in panel a, and as a ribbon diagram of the structure in panel b. The amino-terminal domain, D_1, is similar in structure to an immunoglobulin V domain. The second domain, D_2, although clearly related to an immunoglobulin domain, is different from both V and C domains and has been termed a C2 domain. The first two domains of CD4 form a rigid rodlike structure that is linked to the two carboxy-terminal domains by a flexible link. The binding site for MHC class II molecules is thought to involve both the D_1 and D_2 domains. The CD8 molecule (panels a and c) is a heterodimer of an α and a β chain covalently linked by a disulfide bond; an alternative form of CD8 exists as a homodimer of α chains. The heterodimer is depicted in panel a, while the ribbon diagram in panel c is of the homodimer. CD8α and CD8β chains have very similar structures, each having a single domain resembling an immunoglobulin V domain and a stretch of polypeptide chain, believed to be in a relatively extended conformation, that anchors the V-like domain to the cell membrane.

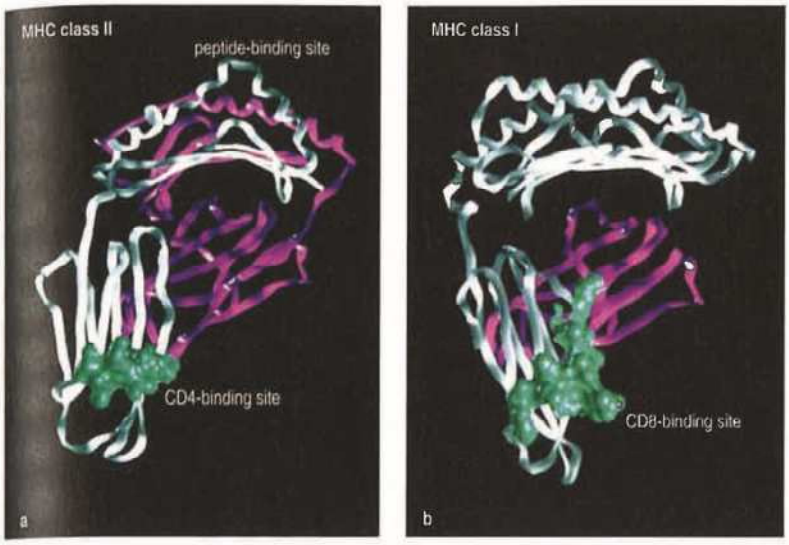


Fig. 3.16 The binding sites for CD4 and CD8 on MHC class II and class I molecules lie in the immunoglobulin-like domains. The binding sites for CD4 and CD8 on the MHC class II and class I molecules, respectively, lie in the immunoglobulin-like domains nearest to the membrane and distant from the peptide-binding cleft. In panel a, the binding site for CD4 is shown as a bright green surface. It lies at the base of the β_2 domain of an MHC class II molecule and is distant from the peptide-binding site at the top of the molecule. The α chain of the MHC class II molecule is purple, and the β chain is white. In panel b, the binding site for CD8 is also shown as a green surface, at the base of the α_3 domain of the MHC class I molecule. The α chain of the class I molecule is white, and the β_2-microglobulin is purple. Photographs courtesy of C. Thorpe.

tissues of the body (see Section 3-13). CD4 and CD8 were known as markers for different functional sets of T cells for some time before it became clear that they play an important part in the direct recognition of MHC class II and MHC class I molecules, respectively. CD4 binds to the MHC class II molecule and CD8 to the MHC class I molecule. During antigen recognition, depending on the type of T-cell, CD4 or CD8 molecules associate on the T-cell surface with the T-cell receptor and bind to invariant sites on the MHC portion of the composite MHC:peptide ligand. This binding is required for the T cell to make an effective response, and so CD4 and CD8 are called **co-receptors.**

CD4 is a single-chain molecule composed of four immunoglobulin-like domains (Fig. 3.15). The first two domains (D_1 and D_2) of the CD4 molecule are packed tightly together to form a rigid rod some 60 Å long, which is joined by a flexible hinge to a similar rod formed by the third and fourth domains (D_3 and D_4). CD4 binds MHC class II molecules through a region that is located mainly on a lateral face of the first domain, D_1. Because CD4 binds to a site on the β_2 domain of the MHC class II molecule that is well away from the site where the T-cell receptor binds (Fig. 3.16a), the CD4 molecule and the T-cell receptor can bind the same peptide:MHC class II complex. CD4 interacts strongly with a cytoplasmic tyrosine kinase called **Lck**, and can deliver this tyrosine kinase into close proximity with the signaling components of the T-cell receptor complex. This results in enhancement of the signal that is generated when the T-cell receptor binds its peptide:MHC class II ligand, as we will discuss further in Chapter 6. When CD4 and the T-cell receptor can simultaneously bind to the same MHC class II:peptide complex, the sensitivity of a T cell to antigen presented by MHC class II molecules is markedly increased; the T-cell in this case requires 100-fold less antigen for activation.

CD4 binding to an MHC class II molecule on its own is weak, and it is not clear whether such binding would be able to transmit a signal to the interior of the T cell. As shown in Fig. 3.17, CD4 can form homodimers through a site in the D_4 domain, which leaves the MHC-binding site free to interact with an MHC class II molecule. Thus, the CD4 dimer could cross-link two MHC class II molecules and thus the two T-cell receptors bound to them. Whether the dimerization of CD4 is important in its co-receptor function is not known at present.

Fig. 3.17 CD4 is capable of forming dimers. The structure of the extracellular domains of the CD4 molecule has been determined by X-ray crystallography. Two molecules of CD4 can interact with each other through their D_4 domains, forming homodimers. The site that binds MHC class II molecules remains available in such dimers.

Fig. 3.18 CD8 binds to a site on MHC class I molecules distant from that to which the T-cell receptor binds. The relative positions of the T-cell receptor and CD8 molecules bound to the same MHC class I molecule can be seen in this hypothetical reconstruction of the interaction of an MHC class I molecule (the α chain is shown in green; β_2-microglobulin (dull yellow) can be seen faintly in the background) with a T-cell receptor and CD8. The α and β chains of the T-cell receptor are shown in pink and purple, respectively. The CD8 structure is that of a CD8α homodimer, but is colored to represent the likely orientation of the subunits in the heterodimer, with the CD8α subunit in red and the CD8β subunit in blue. Photograph courtesy of G. Gao.

Although CD4 and CD8 both function as co-receptors, their structures are quite distinct. The CD8 molecule is a disulfide-linked heterodimer consisting of an α and a β chain, each containing a single immunoglobulin-like domain linked to the membrane by a segment of extended polypeptide chain (see Fig. 3.15). This segment is extensively glycosylated, which is thought to be important in maintaining this polypeptide in an extended conformation and protecting it from cleavage by proteases. CD8α chains can also form homodimers, although these are not found when the CD8β chains are present.

CD8 binds weakly to an invariant site in the α_3 domain of an MHC class I molecule (Fig. 3.16b), which is equivalent to the site in MHC class II molecules to which CD4 binds. Although only the interaction of the CD8α homodimer with MHC class I is so far known in detail, it is clear from this that the MHC class I binding site of the CD8 α:β heterodimer will be formed by the interaction of the CD8α and β chains. In addition, CD8 (most probably through the α chain) interacts with residues in the base of the α_2 domain of the MHC class I molecule. Binding in this way, CD8 leaves the upper surface of the MHC class I molecule exposed and free to interact simultaneously with a T-cell receptor, as shown in Fig. 3.18. Like CD4, CD8 also binds Lck through the cytoplasmic tail of the α chain and brings it into close proximity with the T-cell receptor. And as with CD4, the presence of CD8 increases the sensitivity of T cells to antigen presented by MHC class I molecules by about 100-fold. Thus, CD4 and CD8 have similar functions and bind to the same approximate location in MHC class I and MHC class II molecules even though the structures of the two co-receptor proteins are only distantly related.

3-13 The two classes of MHC molecule are expressed differentially on cells.

MHC class I and MHC class II molecules have a distinct distribution among cells that reflects the different effector functions of the T cells that recognize them (Fig. 3.19). MHC class I molecules present peptides from pathogens, commonly viruses, to CD8 cytotoxic T cells, which are specialized to kill any cell that they specifically recognize. As viruses can infect any nucleated cell, almost all such cells express MHC class I molecules, although the level of constitutive expression varies from one cell type to the next. For example, cells of the immune system express abundant MHC class I on their surface, whereas liver cells (hepatocytes) express relatively low levels (see Fig. 3.19). Nonnucleated cells, such as mammalian red blood cells, express little or no MHC class I, and thus the interior of red blood cells is a site in which an infection can go undetected by cytotoxic T cells. As red blood cells cannot support viral replication, this is of no great consequence for viral infection, but it may be the absence of MHC class I that allows the *Plasmodium* species that cause malaria to live in this privileged site.

In contrast, the main function of the CD4 T cells that recognize MHC class II molecules is to activate other effector cells of the immune system. Thus MHC class II molecules are normally found on B lymphocytes, dendritic cells, and macrophages—cells that participate in immune responses—but not on other tissue cells (see Fig. 3.19). When CD4 T cells recognize peptides bound to MHC class II molecules on B cells, they stimulate the B cells to produce antibody Likewise, CD4 T cells recognizing peptides bound to MHC class II molecules on macrophages activate these cells to destroy the pathogens in their vesicles We shall see in Chapter 8 that MHC class II molecules are also expressed on specialized antigen-presenting cells in lymphoid tissues where naive T cells encounter antigen and are first activated. The expression of both MHC class I and MHC class II molecules is regulated by cytokines, in particular interferons, released in the course of immune responses. Interferon-γ (IFN-γ), for

Tissue	MHC class I	MHC class II
Lymphoid tissues		
T cells	+++	+*
B cells	+++	+++
Macrophages	+++	++
Other antigen-presenting cells (eg Langerhans' cells)	+++	+++
Epithelial cells of the thymus	+	+++
Other nucleated cells		
Neutrophils	+++	–
Hepatocytes	+	–
Kidney	+	–
Brain	+	–†
Non-nucleated cells		
Red blood cells	–	–

Fig. 3.19 The expression of MHC molecules differs between tissues. MHC class I molecules are expressed on all nucleated cells, although they are most highly expressed in hematopoietic cells. MHC class II molecules are normally expressed only by a subset of hematopoietic cells and by thymic stromal cells, although they may be expressed by other cell types on exposure to the inflammatory cytokine interferon-γ. *In humans, activated T cells express MHC class II molecules, whereas in mice, all T cells are MHC class II-negative. † In the brain, most cell types are MHC class II-negative but microglia, which are related to macrophages, are MHC class II-positive.

example, increases the expression of MHC class I and MHC class II molecules, and can induce the expression of MHC class II molecules on certain cell types that do not normally express them. Interferons also enhance the antigen-presenting function of MHC class I molecules by inducing the expression of key components of the intracellular machinery that enables peptides to be loaded onto the MHC molecules.

3-14 The two classes of MHC molecule have distinct subunit structures but similar three-dimensional structures.

The two classes of MHC molecule differ from each other in their structure and also have different distributions on the cells of the body. Their different structures enable the two classes of MHC molecules to serve distinct functions in antigen presentation, binding peptides from different intracellular sites and activating different subsets of T cells, as we will see in Chapter 5. Despite their differences in subunit structure, however, MHC class I and class II molecules are closely related in overall structure. In both classes, the two paired protein domains nearest the membrane resemble immunoglobulin domains, whereas the two domains distal to the membrane fold together to create a long cleft, or groove, which is the site at which a peptide binds. Purified peptide:MHC class I and peptide:MHC class II complexes have been characterized structurally, allowing us to describe in detail both the MHC molecules themselves and the way in which they bind peptides.

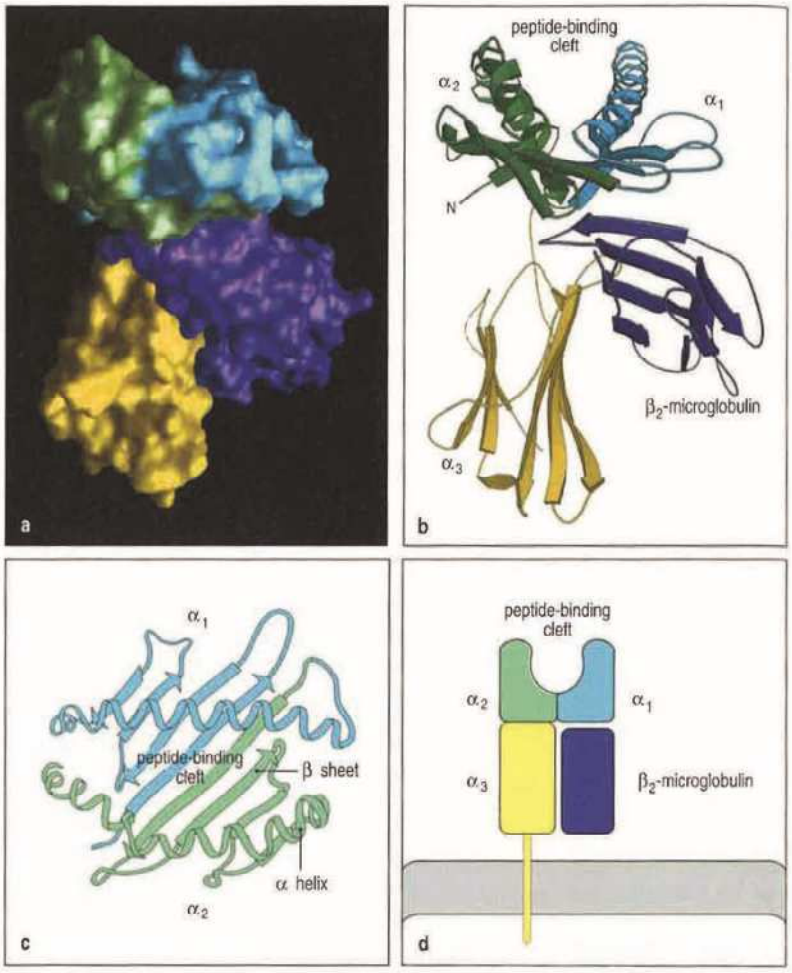


Fig. 3.20 The structure of an MHC class I molecule determined by X-ray crystallography. Panel a shows a computer graphic representation of a human MHC class I molecule, HLA-A2, which has been cleaved from the cell surface by the enzyme papain. The surface of the molecule is shown, colored according to the domains shown in panels b–d and described below. Panels b and c show a ribbon diagram of that structure. Shown schematically in panel d, the MHC class I molecule is a heterodimer of a membrane-spanning α chain (molecular weight 43 kDa) bound noncovalently to β_2-microglobulin (12 kDa), which does not span the membrane. The α chain folds into three domains: α_1, α_2, and α_3. The α_3 domain and β_2-microglobulin show similarities in amino acid sequence to immunoglobulin C domains and have similar folded structures, whereas the α_1 and α_2 domains fold together into a single structure consisting of two segmented α helices lying on a sheet of eight antiparallel β strands. The folding of the α_1 and α_2 domains creates a long cleft or groove, which is the site at which peptide antigens bind to the MHC molecules. The transmembrane region and the short stretch of peptide that connects the external domains to the cell surface are not seen in panels a and b as they have been removed by the papain digestion. As can be seen in panel c, looking down on the molecule from above, the sides of the cleft are formed from the inner faces of the two α helices; the β-pleated sheet formed by the pairing of the α_1 and α_2 domains creates the floor of the cleft. We shall use the schematic representation in panel d throughout this text.

MHC class I structure is outlined in Fig. 3.20. MHC class I molecules consist of two polypeptide chains, a larger α chain encoded in the MHC genetic locus, and a smaller noncovalently associated chain, **β_2-microglobulin**, which is not polymorphic and is not encoded in the MHC locus. Only the class I α chain spans the membrane. The complete molecule has four domains, three formed from the MHC-encoded α chain, and one contributed by β_2-microglobulin. The α_3 domain and β_2-microglobulin have a folded structure that closely resembles that of an immunoglobulin domain. The most remarkable feature of MHC class I molecules is the structure of the folded α_1 and α_2 domains. These two domains form the walls of a cleft on the surface of the molecule; this is the site of peptide binding. They also are sites of polymorphisms that determine T-cell antigen recognition (see Chapter 5).

An MHC class II molecule consists of a noncovalent complex of two chains, α and β, both of which span the membrane (Fig. 3.21). The MHC class II α and β chains are both encoded within the MHC. The crystallographic structure of the MHC class II molecule shows that it is folded very much like the MHC class I molecule. The major differences lie at the ends of the peptide-binding cleft, which are more open in MHC class II molecules compared with MHC

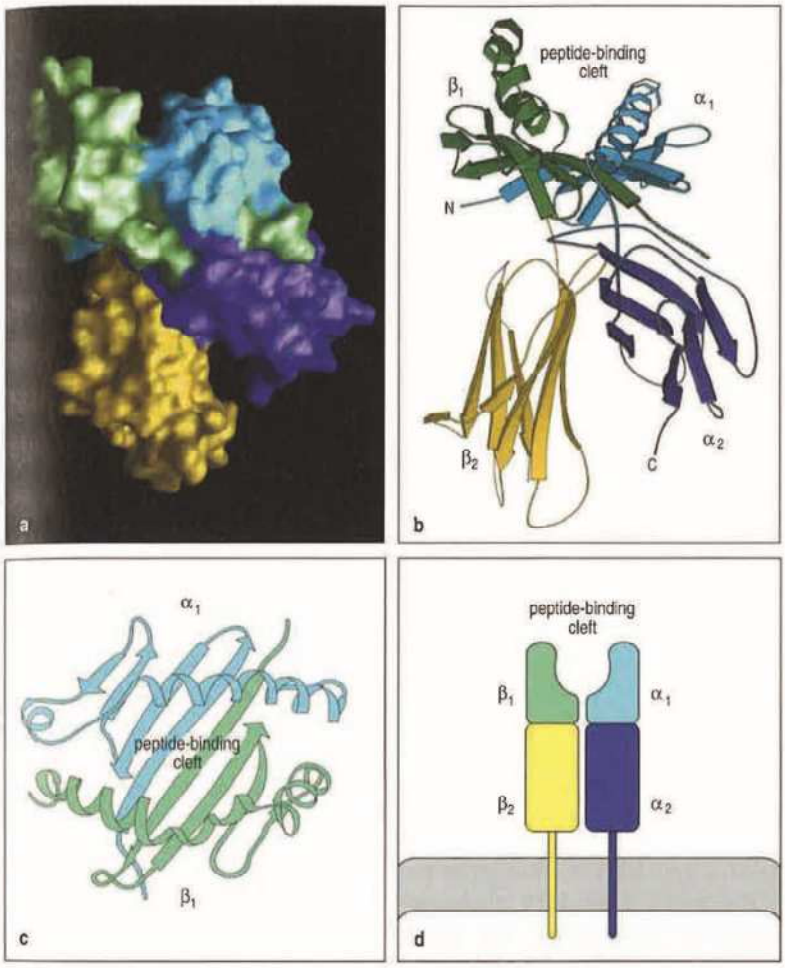


Fig. 3.21 MHC class II molecules resemble MHC class I molecules in overall structure. The MHC class II molecule is composed of two transmembrane glycoprotein chains, α (34 kDa) and β (29 kDa), as shown schematically in panel d. Each chain has two domains, and the two chains together form a compact four-domain structure similar to that of the MHC class I molecule (compare with panel d of Fig. 3.20). Panel a shows a computer graphic representation of the surface of the MHC class II molecule, in this case the human protein HLA-DR1, and panel b shows the equivalent ribbon diagram. The α_2 and β_2 domains, like the α_3 and β_2-microglobulin domains of the MHC class I molecule, have amino acid sequence and structural similarities to immunoglobulin C domains; in the MHC class II molecule, the two domains forming the peptide-binding cleft are contributed by different chains and are therefore not joined by a covalent bond (see panels c and d). Another important difference, not apparent in this diagram, is that the peptide-binding groove of the MHC class II molecule is open at both ends.

class I molecules. The main consequence of this is that the ends of a peptide bound to an MHC class I molecule are substantially buried within the molecule, whereas the ends of peptides bound to MHC class II molecules are not. Again, the sites of major polymorphism are located in the peptide-binding cleft, which in the case of an MHC class II molecule are formed by the α_1, and β_1 domains (see Chapter 5).

In both MHC class I and class II molecules, bound peptides are sandwiched between the two α-helical segments of the MHC molecule (Fig. 3.22). The T-cell receptor interacts with this compound ligand, making contacts with both the MHC molecule and with the peptide fragment of antigen.

3-15 Peptides are stably bound to MHC molecules, and also serve to stabilize the MHC molecule on the cell surface.

An individual can be infected by a wide variety of different pathogens the proteins of which will not generally have peptide sequences in common. If T cells are to be alerted to all possible infections, then the MHC molecules on each cell (both class I and class II) must be able to bind stably to many different peptides. This behavior is quite distinct from that of other peptide-binding receptors, such as those for peptide hormones, which usually bind

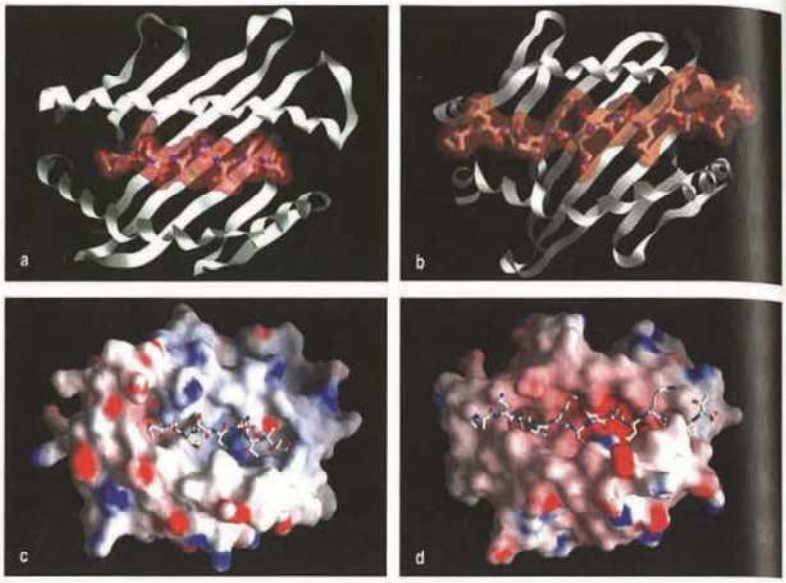


Fig. 3.22 MHC molecules bind peptides tightly within the cleft. When MHC molecules are crystallized with a single synthetic peptide antigen, the details of peptide binding are revealed. In MHC class I molecules (panels a and c) the peptide is bound in an elongated conformation with both ends tightly bound at either end of the cleft. In the case of MHC class II molecules (panels b and d), the peptide is also bound in an elongated conformation but the ends of the peptide are not tightly bound and the peptide extends beyond the cleft. The upper surface of the peptide:MHC complex is recognized by T cells, and is composed of residues of the MHC molecule and the peptide. In representations c and d, the electrostatic potential of the MHC molecule surface is shown, with blue areas indicating a positive potential and red a negative potential.

only a single type of peptide. The crystal structures of peptide:MHC complexes have helped to show how a single binding site can bind peptides with high affinity while retaining the ability to bind a wide variety of different peptides.

An important feature of the binding of peptides to MHC molecules is that the peptide is bound as an integral part of the MHC molecule's structure, and MHC molecules are unstable when peptides are not bound. The stability of peptide binding is important, because otherwise, peptide exchanges occurring at the cell surface would prevent peptide:MHC complexes from being reliable indicators of infection or of uptake of specific antigen. As a result of this stability, when MHC molecules are purified from cells, their bound peptides co-purify with them, and this has enabled the peptides bound by specific MHC molecules to be analyzed. The peptides are released from the MHC molecules by denaturing the complex in acid, and can then be purified and sequenced. Pure synthetic peptides can also be incorporated into previously empty MHC molecules and the structure of the complex determined, revealing details of the contacts between the MHC molecule and the peptide. From the sequences of peptides bound to specific MHC molecules, combined with structural analysis of the peptide:MHC complex, a detailed picture of the binding interactions has been built up. We will first discuss the peptide-binding properties of MHC class I molecules.

3-16 MHC class I molecules bind short peptides of 8–10 amino acids by both ends.

The binding of a peptide in the peptide-binding cleft of an MHC class I molecule is stabilized at both ends by contacts between atoms in the free amino and carboxy termini of the peptide and invariant sites that are found at each end of the cleft of all MHC class I molecules (Fig. 3.23). These contacts are thought to be the main stabilizing contacts for peptide:MHC class I complexes because synthetic peptide analogues lacking terminal amino and carboxyl groups fail to bind stably to MHC class I molecules. Other residues in the peptide serve as additional anchors. Peptides that bind to MHC class I molecules are usually 8–10 amino acids long. The peptide lies in an elongated conformation along the groove; variations in peptide length appear to be

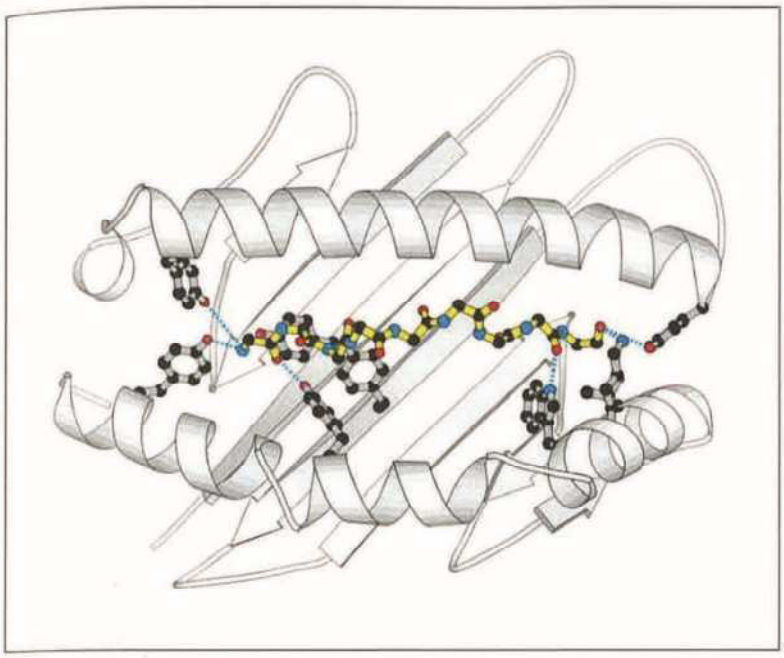


Fig. 3.23 Peptides are bound to MHC class I molecules by their ends. MHC class I molecules interact with the backbone of a bound peptide (shown in yellow) through a series of hydrogen bonds and ionic interactions (shown as dotted blue lines) at each end of the peptide. The amino terminus of the peptide is to the left; the carboxy terminus to the right. Black circles are carbon atoms; red are oxygen; blue are nitrogen. The amino acid residues in the MHC molecule that form these bonds are common to all MHC class I molecules and their side chains are shown in full (in gray) upon a ribbon diagram of the MHC class I groove. A cluster of tyrosine residues common to all MHC class I molecules forms hydrogen bonds to the amino terminus of the bound peptide, while a second cluster of residues forms hydrogen bonds and ionic interactions with the peptide backbone at the carboxy terminus and with the carboxy terminus itself.

accommodated, in most cases, by a kinking in the peptide backbone. However, two examples of MHC class I molecules where the peptide is able to extend out of the groove at the carboxy terminus suggest that some length variation may also be accommodated in this way.

These interactions give all MHC class I molecules their broad peptide-binding specificity. In addition, MHC molecules are highly polymorphic. There are hundreds of different versions, or alleles, of the MHC class I genes in the human population as a whole, and each individual carries only a small selection of them. The main differences between the allelic MHC variants are found at certain sites in the peptide-binding cleft, resulting in different amino acids in key peptide interaction sites in the different MHC variants. The consequence of this is that the different MHC variants preferentially bind different peptides. The peptides that can bind to a given MHC variant have the same or very similar amino acid residues at two or three particular positions along the peptide sequence. The amino acid side chains at these positions insert into pockets in the MHC molecule that are lined by the polymorphic amino acids. Because the binding of these side chains anchors the peptide to the MHC molecule, the peptide residues involved have been called **anchor residues**. Both the position and identity of these anchor residues can vary, depending on the particular MHC class I variant that is binding the peptide. However, most peptides that bind to MHC class I molecules have a hydrophobic (or sometimes basic) anchor residue at the carboxy terminus (Fig. 3.24). Changing an anchor residue can prevent the peptide from binding and, conversely, most synthetic peptides of suitable length that contain these anchor residues will bind the appropriate MHC class I molecule, in most cases irrespective of the amino acids at other positions in the peptide. These features of peptide binding enable an individual MHC class I molecule to bind a wide variety of different peptides, yet allow different MHC class I allelic variants to bind different sets of peptides.

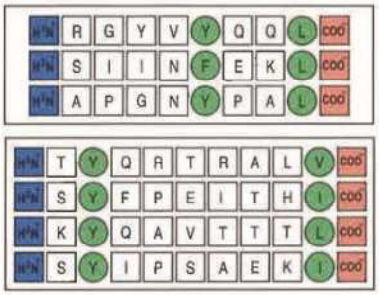


Fig. 3.24 Peptides bind to MHC molecules through structurally related anchor residues. Peptides eluted from two different MHC class I molecules are shown. The anchor residues (green) differ for peptides that bind different alleles of MHC class I molecules but are similar for all peptides that bind to the same MHC molecule. The upper and lower panels show peptides that bind to two different alleles of MHC class I molecules. The anchor residues that bind a particular MHC molecule need not be identical, but are always related (for example, phenylalanine (F) and tyrosine (Y) are both aromatic amino acids, whereas valine (V), leucine (L), and isoleucine (I) are all large hydrophobic amino acids). Peptides also bind to MHC class I molecules through their amino (blue) and carboxy (red) termini.

3-17 The length of the peptides bound by MHC class II molecules is not constrained.

Peptide binding to MHC class II molecules has also been analyzed by elution of bound peptides and by X-ray crystallography, and differs in several ways from peptide binding to MHC class I molecules. Peptides that bind to MHC class II molecules are at least 13 amino acids long and can be much longer. The clusters of conserved residues that bind the two ends of a peptide in MHC class I molecules are not found in MHC class II molecules, and the ends of the peptide are not bound. Instead, the peptide lies in an extended conformation along the MHC class II peptide-binding groove. It is held in this groove both by peptide side chains that protrude into shallow and deep pockets lined by polymorphic residues, and by interactions between the peptide backbone and side chains of conserved amino acids that line the peptide-binding cleft in all MHC class II molecules (Fig. 3.25). Although there are fewer crystal structures of MHC class II-bound peptides than of MHC class I, the available data show that amino acid side chains at residues 1, 4, 6, and 9 of an MHC class II-bound peptide can be held in these binding pockets.

The binding pockets of MHC class II molecules are more permissive in their accommodation of different amino acid side chains than are those of the MHC class I molecule, making it more difficult to define anchor residues and predict which peptides will be able to bind particular MHC class II molecules (Fig. 3.26). Nevertheless, by comparing the sequences of known binding peptides, it is usually possible to detect a pattern of permissive amino acids for each of the different alleles of MHC class II molecules, and to model how the amino acids of this peptide sequence motif will interact with the amino acids that make up the peptide-binding cleft in the MHC class II molecule. Because the peptide is bound by its backbone and allowed to emerge from both ends of the binding groove there is, in principle, no upper limit to the length of peptides that could bind to MHC class II molecules. However, it appears that longer peptides bound to MHC class II molecules are trimmed

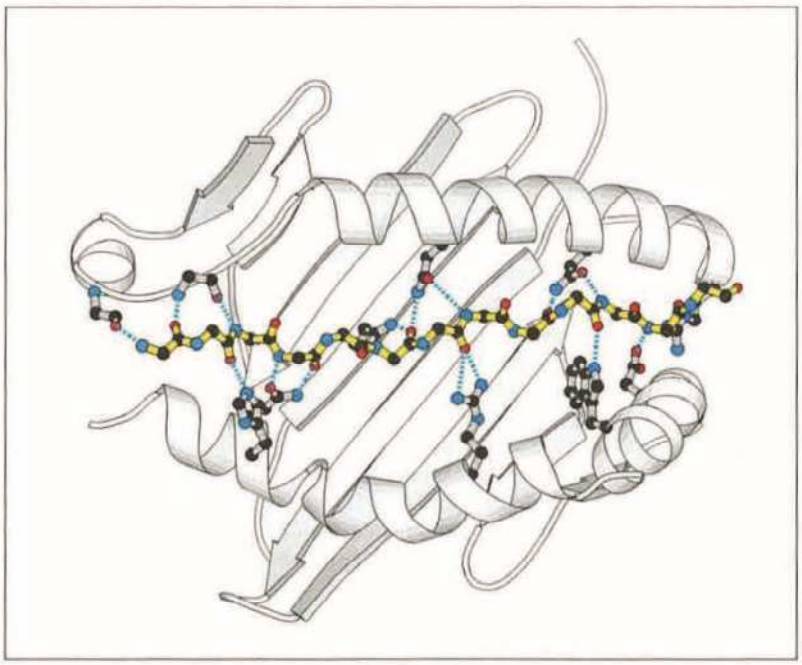


Fig. 3.25 Peptides bind to MHC class II molecules by interactions along the length of the binding groove. A peptide (yellow; shown as the peptide backbone only, with the amino terminus to the left and the carboxy terminus to the right), is bound by an MHC class II molecule through a series of hydrogen bonds (dotted blue lines) that are distributed along the length of the peptide. The hydrogen bonds toward the amino terminus of the peptide are made with the backbone of the MHC class II polypeptide chain, whereas throughout the peptide's length bonds are made with residues that are highly conserved in MHC class II molecules. The side chains of these residues are shown in gray upon the ribbon diagram of the MHC class II groove.

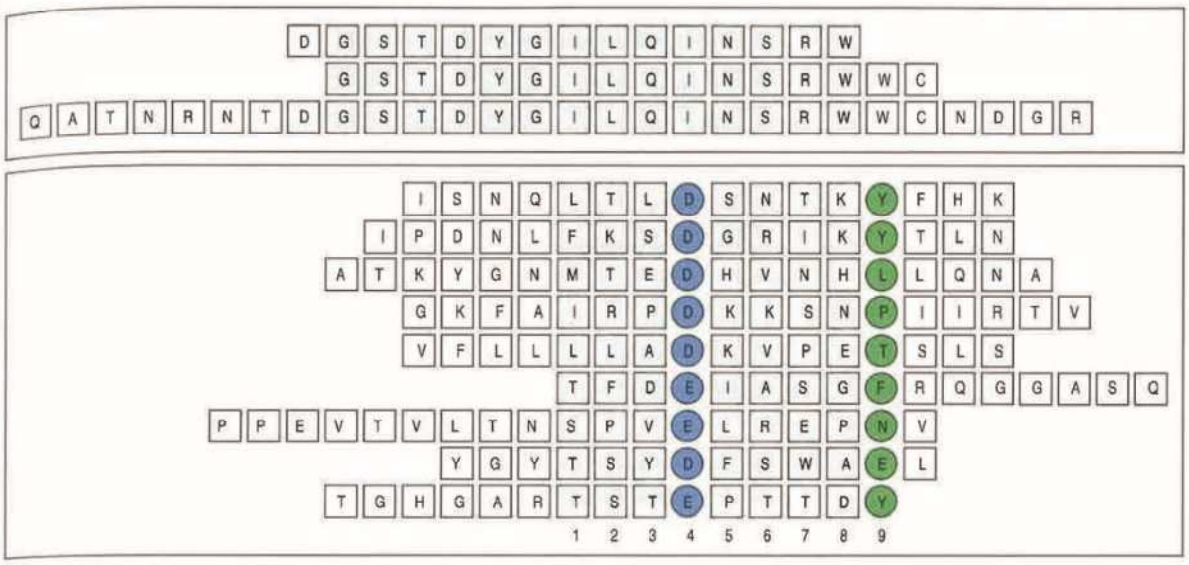


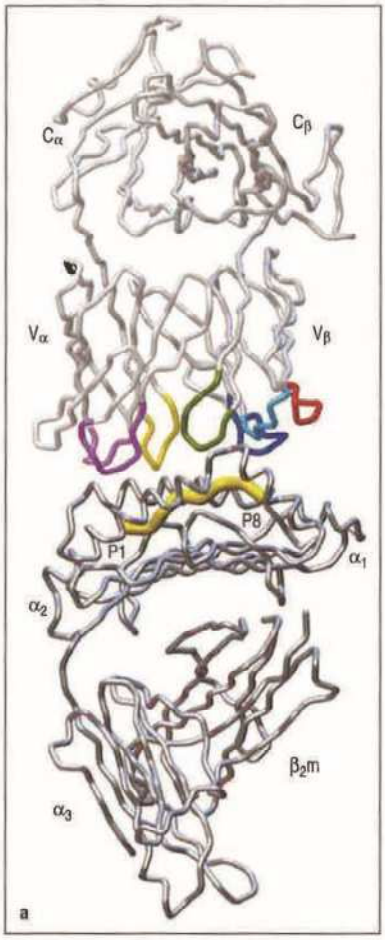
Fig. 3.26 Peptides that bind MHC class II molecules are variable in length and their anchor residues lie at various distances from the ends of the peptide. The sequences of a set of peptides that bind to the mouse MHC class II A^k allele are shown in the upper panel. All contain the same core sequence but differ in length. In the lower panel, different peptides binding to the human MHC class II allele HLA-DR3 are shown. The lengths of these peptides can vary, and so by convention the first anchor residue is denoted as residue 1. Note that all of the peptides share a negatively charged residue (aspartic acid (D) or glutamic acid (E)) in the P4 position (blue) and tend to have a hydrophobic residue (for example, tyrosine (Y), leucine (L), proline (P), phenylalanine (F)) in the P9 position (green).

by peptidases to a length of 13–17 amino acids in most cases. Like MHC class I molecules, MHC class II molecules that lack bound peptide are unstable, but the critical stabilizing interactions that the peptide makes with the MHC class II molecule are not yet known.

3-18 The crystal structures of several MHC:peptide:T-cell receptor complexes all show the same T-cell receptor orientation over the MHC:peptide complex.

At the time that the first X-ray crystallographic structure of a T-cell receptor was published, a structure of the same T-cell receptor bound to a peptide:MHC class I ligand was also produced. This structure (Fig. 3.27), which had been forecast by site-directed mutagenesis of the MHC class I molecule, showed the T-cell receptor aligned diagonally over the peptide and the peptide-binding groove, with the T-cell receptor α chain lying over the α_2 domain and the amino-terminal end of the bound peptide, the T-cell receptor β chain lying over the α_1 domain and the carboxy-terminal end of the peptide, with the CDR3 loops of both T-cell receptor α and T-cell receptor β meeting over the central amino acids of the peptide. The T-cell receptor is threaded through a valley between the two high points on the two surrounding α helices that form the walls of the peptide-binding cleft.

Analysis of other MHC class I:peptide:T-cell receptor complexes and of the single example so far of an MHC class II:peptide:T-cell receptor complex (Fig. 3.28) shows that all of them have a very similar orientation, particularly for the V_α domain, although some variability does occur in the location and orientation of the V_β domain. In this orientation, the V_α domain makes contact primarily with the amino terminus of the bound peptide, whereas the V_β domain contacts primarily the carboxy terminus of the bound peptide. Both



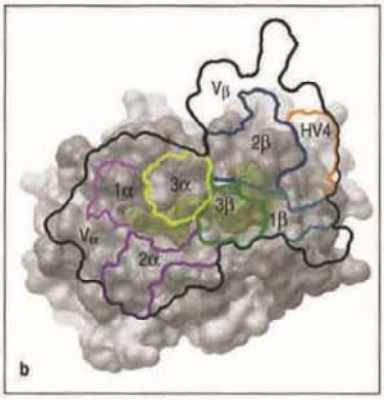


Fig. 3.27 The T-cell receptor binds to the MHC:peptide complex. Panel a: the T-cell receptor binds to the top of the MHC:peptide complex, straddling, in the case of the class I molecule shown here, both the α_1 and α_2 domain helices. The CDRs of the T-cell receptor are indicated in color; the CDR1 and CDR2 loops of the β chain in light and dark blue, respectively; and the CDR1 and CDR2 loops of the α chain in light and dark purple, respectively. The α chain CDR3 loop is in yellow while the β chain CDR3 loop is in green. The β chain HV4 loop is orange. Panel b: the outline of the T-cell receptor antigen-binding site (thick black line) is superimposed upon the top surface of the MHC:peptide complex (the peptide is shaded dull yellow). The T-cell receptor lies diagonally across the MHC:peptide complex, with the α and β CDR3 loops of the T-cell receptor (3α, 3β, yellow and green, respectively) contacting the center of the peptide. The α chain CDR1 and CDR2 loops (1α, 2α, light and dark purple, respectively) contact the MHC helices at the amino terminus of the bound peptide, whereas the β chain CDR1 and CDR2 loops (1β, 2β, light and dark blue, respectively) make contact with the helices at the carboxy terminus of the bound peptide. Courtesy of I.A. Wilson.

chains also interact with the α helices of the MHC class I molecule (see Fig. 3.27). The T-cell receptor contacts are not symmetrically distributed over the MHC molecule, so whereas the V_α CDR1 and CDR2 loops are in close contact with the helices of the MHC:peptide complex around the amino terminus of the bound peptide, the β-chain CDR1 and CDR2 loops, which interact with the complex at the carboxy terminus of the bound peptide, have variable contributions to the binding. This suggests that the V_α contacts are responsible for the conserved orientation of the T-cell receptor on the MHC:peptide complex.

Comparison of the three-dimensional structure of the T-cell receptor to that of the same T-cell receptor complexed to its MHC-peptide ligand could address the question of whether the T-cell receptor, like some other receptors, undergoes a conformational change, or 'induced fit,' in its three-dimensional structure when it binds its specific ligand. To date, there is no certain answer, owing to the limitations of the available structures. For one thing, all the T-cell receptors analyzed to date have either been bound to ligands that do not produce activation, or are bound to ligands that can activate them, but the comparable unliganded receptor structures are not available. Also, the crystals of the T-cell receptor are all formed at 0 °C or below, which locks the receptor into a single conformation. However, subtly different peptides can have strikingly different effects when the same T cell recognizes either of the two peptides complexed with MHC. This could be due to differences in how T-cell receptor conformation is altered by binding the two related yet different ligands. Recent evidence also suggests that the temperature at which the T-cell receptor binds to a particular peptide:MHC complex makes a large difference in the extent of T-cell receptor aggregation; protein conformation is affected by temperature, and so these differences may well result from a conformational change.

From an examination of these structures it is hard to predict whether the main binding energy is contributed by T-cell receptor contacts with the bound peptide, or by T-cell receptor contacts with the MHC molecule. It is known that alterations as simple as changing a leucine to isoleucine in the peptide are sufficient to alter the T-cell response from strong killing to absolutely no response at all. Studies show that mutations of single residues in the presenting MHC molecules can have the same effect. Thus, the specificity of T-cell recognition involves both the peptide and its presenting MHC molecule. This dual specificity underlies the MHC restriction of T-cell responses, a phenomenom that was observed long before the peptide-binding properties of MHC molecules were known. We will recount the story of how MHC restriction was discovered when we return to the issue of how MHC polymorphism affects antigen recognition by T cells in Chapter 5.

Another consequence of this dual specificity is a need for T-cell receptors to be able to interact appropriately with the antigen-presenting surface of MHC molecules. It appears that there is some inherent specificity for MHC molecules encoded in the T-cell receptor genes, as well as selection during T-cell development for a repertoire of receptors able to interact appropriately with the particular MHC molecules present in that individual. We will be discussing the evidence for this in Chapter 7.

3-19 A distinct subset of T cells bears an alternative receptor made up of γ and δ chains.

During the search for the gene for the T-cell receptor α chain, another T-cell receptorlike gene was unexpectedly discovered. This gene was named T-cell receptor γ, and its discovery led to a search for further T-cell receptor genes. Another receptor chain was identified using antibody to the predicted sequence of the γ chain and was called the δ chain. It was soon discovered that a minority population of T cells bore a distinct type of T-cell receptor made up of γ:δ heterodimers rather than α:β heterodimers. The development of these cells is described in Sections 7-13 and 7-14.

To date, there is no crystallographic structure of a γ:δ T-cell receptor, although it is expected to be similar in shape to α:β T-cell receptors. γ:δ T-cell receptors may be specialized to bind certain kinds of ligands, including heat-shock proteins and nonpeptide ligands such as mycobacterial lipid antigens. It seems likely that γ:δ T-cell receptors are not restricted by the 'classical' MHC class I and class II molecules. They may bind the free antigen, much as immunoglobulins do, and/or they may bind to peptides or other antigens presented by nonclassical MHC-like molecules. These are proteins that resemble MHC class I molecules but are relatively nonpolymorphic. We still know little about how γ:δ T-cell receptors bind antigen and thus how these cells function, and what their role is in immune responses. The structure and rearrangement of the genes for γ:δ T-cell receptors is covered in Sections 4-13 and 7-13 and the functions of γ:δ T cells are considered in Chapter 8.

Summary.

The receptor for antigen on most T cells, the α:β T-cell receptor, is composed of two protein chains, T-cell receptor α and T-cell receptor β, and resembles in many respects a single Fab fragment of immunoglobulin. T-cell receptors are always membrane-bound. α:β T-cell receptors do not recognize antigen in its native state, as do the immunoglobulin receptors of B cells, but recognize a composite ligand of a peptide antigen bound to an MHC molecule. MHC molecules are highly polymorphic glycoproteins encoded by genes in the major histocompatibility complex (MHC). Each MHC molecule binds a wide variety of different peptides, but the different variants each preferentially recognize sets of peptides with particular sequence and physical features. The peptide antigen is generated intracellularly, and bound stably in a peptide-binding cleft on the surface of the MHC molecule. There are two classes of MHC molecules and these are bound in their nonpolymorphic domains by CD8 and CD4 molecules that distinguish two different functional classes of α:β T cells. CD8 binds MHC class I molecules and can bind simultaneously to the same class I MHC:peptide complex being recognized by a T-cell receptor, thus acting as a co-receptor and enhancing the T-cell response; CD4 binds MHC class II molecules and acts as a co-receptor for T-cell receptors that recognise class II MHC:peptide ligands. T-cell receptors interact directly with both the antigenic peptide and polymorphic features of the MHC molecule that displays it, and this dual specificity underlies the

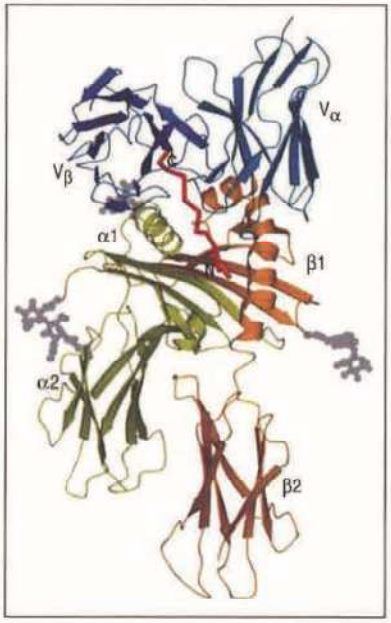


Fig. 3.28 The T-cell receptor interacts with MHC class I and MHC class II molecules in a similar fashion. The structure of a T-cell receptor binding to an MHC class II molecule has been determined, and shows the T-cell receptor binding to an equivalent site, and in an equivalent orientation, to the way that TCRs bind to MHC class I molecules (see Fig. 3.27). The structure of the molecules is shown in a cartoon form, with the MHC class II α and β chains shown in light green and orange respectively. Only the V_α and V_β domains of the T-cell receptor are shown, colored in blue. The peptide is colored red, while carbohydrate residues are indicated in gray. The TCR sits in a shallow saddle formed between the MHC class II α and β chain α-helical regions, at roughly 90° to the long axis of the MHC class II molecule and the bound peptide. Courtesy of E.L. Reinherz.

MHC restriction of T-cell responses. A second type of T-cell receptor, composed of a γ and a δ chain, is structurally similar to the α:β T-cell receptor but appears to bind different ligands, including nonpeptide ligands. It is not thought to be MHC restricted. It is found on a minority population of T cells, the γ:δ T cells, whose biological function is still not clear.

Summary to Chapter 3.

B cells and T cells use different, but structurally similar, molecules to recognize antigen. The antigen-recognition molecules of B cells are immunoglobulins, and are made both as a membrane-bound receptor for antigen, the B-cell receptor, and as secreted antibodies that bind antigens and elicit humoral effector functions. The antigen-recognition molecules of T cells, on the other hand, are made only as cell-surface receptors. Immunoglobulins and T-cell receptors are highly variable molecules, with the variability concentrated in that part of the molecule, the variable (V) region, that binds to antigen Immunoglobulins bind a wide variety of chemically different antigens, whereas the major α:β form of T-cell receptors will recognize only peptide fragments of foreign proteins bound to the MHC molecules that are ubiquitous on cell surfaces.

Binding of antigen by immunoglobulins has chiefly been studied using antibodies. The binding of antibody to its corresponding antigen is highly specific, and this specificity is determined by the shape and physicochemical properties of the antigen-binding site. The part of the antibody that elicits effector functions, once the variable part has bound an antigen, is located at the other end of the molecule from the antigen-binding sites, and is termed the constant region. There are five main functional classes of antibody, each encoded by a different type of constant region. As we will see in Chapter 9, these in turn interact with different components of the immune system to incite an inflammatory response and eliminate the antigen.

The T-cell receptor differs in several respects from the B-cell immunoglobulins. Among the most important of these differences is the absence of a secreted form of the receptor. This reflects the functional differences between T cells and B cells. B cells deal with pathogens and their protein products circulating within the body; secretion of a soluble antigen-recognition molecule by the activated B cell after antigen has been encountered enables them to mop up antigen effectively throughout the extracellular spaces of the body. T cells, on the other hand, are specialized for cell–cell interactions. They either kill cells that are infected with intracellular pathogens and that bear foreign antigenic peptides on their surface, or interact with cells of the immune system that have taken up foreign antigen and are displaying it on the cell surface. They thus have no requirement for a soluble, secreted receptor.

An additional distinctive feature of the T-cell receptor compared with immunoglobulins is that it recognizes a composite ligand made up of the foreign peptide bound to a self MHC molecule. This forces T cells to interact with infected body cells to become activated. Each T-cell receptor is specific for a particular combination of peptide and a self MHC molecule.

MHC molecules are encoded by a family of highly polymorphic genes and, although each individual expresses several, this represents only a small selection of all possible variants. During T-cell development, the T-cell receptor repertoire is selected so that the T cells of each individual recognize antigen only in conjunction with their own MHC molecules. Expression of multiple variant MHC molecules each with a different peptide-binding repertoire helps ensure that T cells from an individual will be able to recognize at least some peptides generated from nearly every pathogen.

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