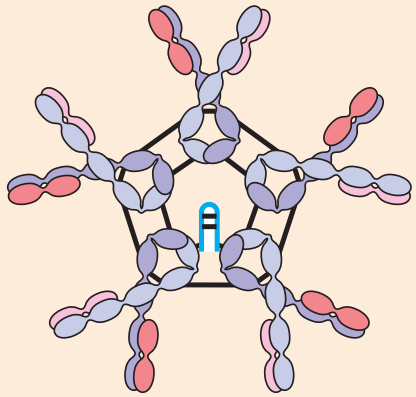
Antibodies:
Structure and Function

chapter 4



IgM, the First Responder

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- Immunoglobulin Fine Structure
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- Antigenic Determinants on Immunoglobulins
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ANTIBODIES ARE THE ANTIGEN-BINDING PROTEINS present on the B-cell membrane and secreted by plasma cells. Membrane-bound antibody confers antigenic specificity on B cells; antigen-specific proliferation of B-cell clones is elicited by the interaction of membrane antibody with antigen. Secreted antibodies circulate in the blood, where they serve as the effectors of humoral immunity by searching out and neutralizing antigens or marking them for elimination. All antibodies share structural features, bind to antigen, and participate in a limited number of effector functions.

The antibodies produced in response to a particular antigen are heterogeneous. Most antigens are complex and contain many different antigenic determinants, and the immune system usually responds by producing antibodies to several epitopes on the antigen. This response requires the recruitment of several clones of B cells. Their outputs are monoclonal antibodies, each of which specifically binds a single antigenic determinant. Together, these monoclonal antibodies make up the polyclonal and heterogeneous serum antibody response to an immunizing antigen.

Basic Structure of Antibodies

Blood can be separated in a centrifuge into a fluid and a cellular fraction. The fluid fraction is the **plasma** and the cellular fraction contains red blood cells, leukocytes, and platelets. Plasma contains all of the soluble small molecules and macromolecules of blood, including fibrin and other proteins required for the formation of blood clots. If the blood or plasma is allowed to clot, the fluid phase that remains is called **serum.** It has been known since the turn of the century that antibodies reside in the serum. The first evidence that antibodies were contained in particular serum protein fractions came from a classic experiment by A. Tiselius and E. A. Kabat, in 1939. They immunized rabbits with the protein ovalbumin (the albumin of egg whites) and then divided the immunized rabbits' serum into two aliquots. Electrophoresis of one serum aliquot revealed four peaks corresponding to albumin and the alpha (α), beta (β), and gamma (γ) globulins. The other serum aliquot was reacted with ovalbumin, and the precipitate that formed was removed; the remaining serum proteins, which did not react with the antigen, were then electrophoresed. A comparison of the electrophoretic profiles of these two serum aliquots revealed that there was a significant drop in the γ-globulin peak in the aliquot that had been reacted with antigen (Figure 4-1). Thus, the **γ-globulin fraction** was identified as containing serum antibodies, which were called **immunoglobulins,** to distinguish them from any other proteins that might be contained in the γ-globulin fraction. The early experiments of Kabat and Tiselius resolved serum proteins into three major nonalbumin peaks—α, β and γ. We now know that although immunoglobulin G (IgG), the main class of antibody molecules, is indeed mostly found in the γ-globulin fraction, significant amounts of it and other important classes of antibody molecules are found in the α and the β fractions of serum.

Antibodies Are Heterodimers

Antibody molecules have a common structure of four peptide chains (Figure 4-2). This structure consists of two identical **light (L) chains,** polypeptides of about 25,000 molecular weight, and two identical **heavy (H) chains,** larger

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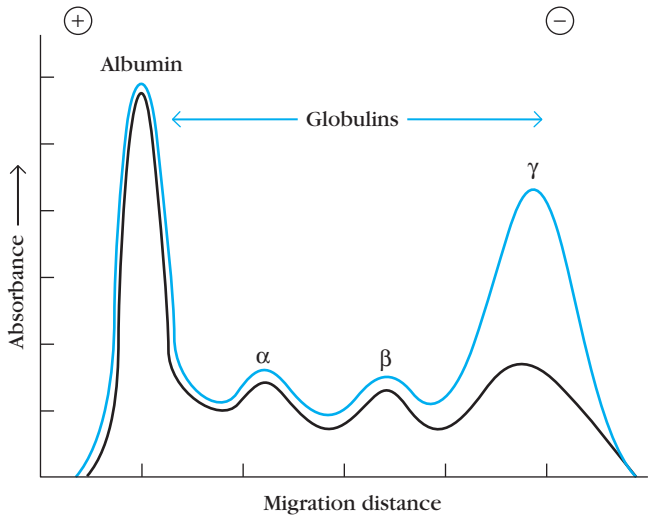

FIGURE 4-1 Experimental demonstration that most antibodies are in the γ-globulin fraction of serum proteins. After rabbits were immunized with ovalbumin (OVA), their antisera were pooled and electrophoresed, which separated the serum proteins according to their electric charge and mass. The blue line shows the electrophoretic pattern of untreated antiserum. The black line shows the pattern of antiserum that was incubated with OVA to remove anti-OVA antibody and then electrophoresed. *[Adapted from A. Tiselius and E. A. Kabat, 1939,* J. Exp. Med. ***69:****119, with copyright permission of the Rockefeller University Press.]*

polypeptides of molecular weight 50,000 or more. Like the antibody molecules they constitute, H and L chains are also called immunoglobulins. Each light chain is bound to a heavy chain by a disulfide bond, and by such noncovalent interactions as salt linkages, hydrogen bonds, and hydrophobic bonds, to form a heterodimer (H-L). Similar noncovalent interactions and disulfide bridges link the two identical heavy and light (H-L) chain combinations to each other to form the basic four-chain $(H\text{-}L)_2$ antibody structure, a dimer of dimers. As we shall see, the exact number and precise positions of these interchain disulfide bonds differs among antibody classes and subclasses.

The first 110 or so amino acids of the amino-terminal region of a light or heavy chain varies greatly among antibodies of different specificity. These segments of highly variable sequence are called *V regions:* V_L in light chains and V_H in heavy. All of the differences in specificity displayed by different antibodies can be traced to differences in the amino acid sequences of V regions. In fact, most of the differences among antibodies fall within areas of the V regions called *complementarity-determining regions (CDRs),* and it is these CDRs, on both light and heavy chains, that constitute the antigen-binding site of the antibody molecule. By contrast, within the same antibody class, far fewer differences are seen when one compares sequences throughout the rest of the molecule. The regions of relatively constant sequence beyond the variable regions have been dubbed C regions, C_L on the light chain and C_H on the heavy chain. Antibodies are glycoproteins; with few exceptions, the sites of attachment for carbohydrates are restricted to the constant region. We do not completely understand the role played by glycosylation of antibodies, but it probably increases the solubility of the molecules. Inappropriate glycosylation, or its absence, affects the rate at which antibodies are cleared from the serum, and decreases the efficiency of interaction between antibody and the complement system and between antibodies and Fc receptors.

Chemical and Enzymatic Methods Revealed Basic Antibody Structure

Our knowledge of basic antibody structure was derived from a variety of experimental observations. When the γ-globulin fraction of serum is separated into high- and low-molecular-weight fractions, antibodies of around 150,000-MW, designated as immunoglobulin G (IgG) are found in the low-molecular-weight fraction. In a key experiment, brief digestion of IgG with the enzyme papain produced three fragments, two of which were identical fragments and a third that was quite different (Figure 4-3). The two identical fragments

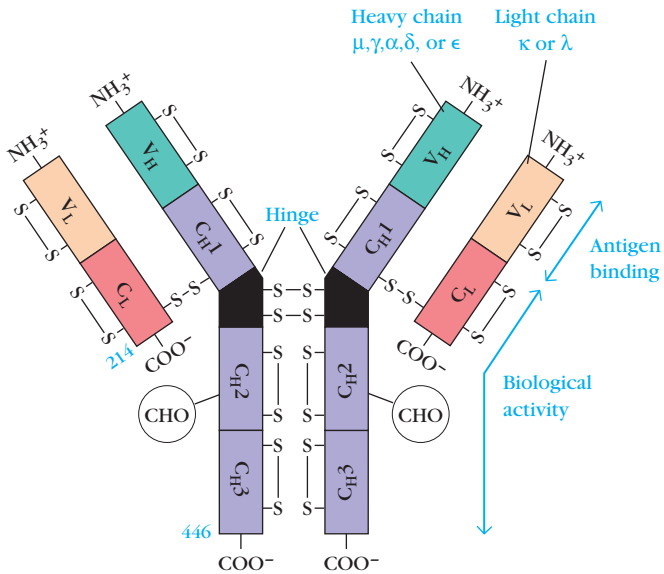

FIGURE 4-2 Schematic diagram of structure of immunoglobulins derived from amino acid sequencing studies. Each heavy and light chain in an immunoglobulin molecule contains an amino-terminal variable (V) region (aqua and tan, respectively) that consists of 100–110 amino acids and differs from one antibody to the next. The remainder of each chain in the molecule—the constant (C) regions (purple and red)—exhibits limited variation that defines the two light-chain subtypes and the five heavy-chain subclasses. Some heavy chains (γ, δ, and α) also contain a proline-rich hinge region (black). The amino-terminal portions, corresponding to the V regions, bind to antigen; effector functions are mediated by the other domains. The μ and ε heavy chains, which lack a hinge region, contain an additional domain in the middle of the molecule.

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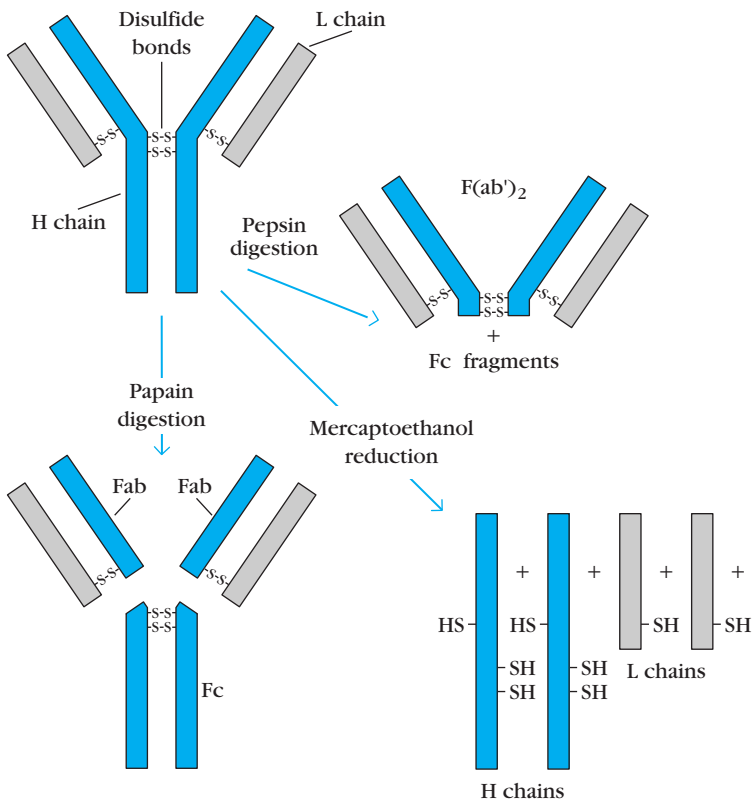

FIGURE 4-3 Prototype structure of IgG, showing chain structure and interchain disulfide bonds. The fragments produced by various treatments are also indicated. Light (L) chains are in gray and heavy (H) chains in blue.

(each with a MW of 45,000), had antigen-binding activity and were called **Fab fragments** ("fragment, antigen binding"). The other fragment (MW of 50,000) had no antigen-binding activity at all. Because it was found to crystallize during cold storage, it was called the **Fc fragment** ("fragment, crystallizable"). Digestion with pepsin, a different proteolytic enzyme, also demonstrated that the antigen-binding properties of an antibody can be separated from the rest of the molecule. Pepsin digestion generated a single 100,000-MW fragment composed of two Fab-like fragments designated the **F(ab′)$_2$ fragment,** which binds antigen. The Fc fragment was not recovered from pepsin digestion because it had been digested into multiple fragments.

A key observation in deducing the multichain structure of IgG was made when the molecule was subjected to mercaptoethanol reduction and alkylation, a chemical treatment that irreversibly cleaves disulfide bonds. If the sample is chromatographed on a column that separates molecules by size following cleavage of disulfide bonds, it is clear that the intact 150,000-MW IgG molecule is, in fact, composed of subunits. Each IgG molecule contains two 50,000-MW polypeptide chains, designated as heavy (H) chains, and two 25,000-MW chains, designated as light (L) chains (see Figure 4-3).

Antibodies themselves were used to determine how the enzyme digestion products—Fab, F(ab′)$_2$, and Fc—were related to the heavy-chain and light-chain reduction products. This question was answered by using antisera from goats that had been immunized with either the Fab fragments or the Fc fragments of rabbit IgG. The antibody to the Fab fragment could react with both the H and the L chains, whereas antibody to the Fc fragment reacted only with the H chain. These observations led to the conclusion that the Fab fragment consists of portions of a heavy and a light chain and that Fc contains only heavy-chain components. From these results, and those mentioned above, the structure of IgG shown in Figure 4-3 was deduced. According to this model, the IgG molecule consists of two identical H chains and two identical L chains, which are linked by disulfide bridges. The enzyme papain cleaves just above the interchain disulfide bonds linking the heavy chains, whereas the enzyme pepsin cleaves just below these bonds, so that the two proteolytic enzymes generate different digestion products. Mercaptoethanol reduction and alkylation allow separation of the individual heavy and light chains.

Obstacles to Antibody Sequencing

Initial attempts to determine the amino acid sequence of the heavy and light chains of antibody were hindered because insufficient amounts of homogeneous protein were available. Although the basic structure and chemical properties of differ-

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ent antibodies are similar, their antigen-binding specificities, and therefore their exact amino acid sequences, are very different. The population of antibodies in the serum γ-globulin fraction consists of a heterogeneous spectrum of antibodies. Even if immunization is done with a hapten-carrier conjugate, the antibodies formed just to the hapten alone are heterogeneous: they recognize different epitopes of the hapten and have different binding affinities. This heterogeneity of serum antibodies made them unsuitable for sequencing studies.

Pure Immunoglobulin Obtained from Multiple Myeloma Patients Made Sequencing Possible

Sequencing analysis finally became feasible with the discovery of **multiple myeloma,** a cancer of antibody-producing plasma cells. The plasma cells in a normal individual are end-stage cells that secrete a single molecular species of antibody for a limited period of time and then die. In contrast, a clone of plasma cells in an individual with multiple myeloma has escaped normal controls on their life span and proliferation and are not end-stage cells; rather, they divide over and over in an unregulated way without requiring any activation by antigen to induce proliferation. Although such a cancerous plasma cell, called a **myeloma cell,** has been transformed, its protein-synthesizing machinery and secretory functions are not altered; thus, the cell continues to secrete molecularly homogeneous antibody. This antibody is indistinguishable from normal antibody molecules but is called **myeloma protein** to denote its source. In a patient afflicted with multiple myeloma, myeloma protein can account for 95% of the serum immunoglobulins. In most patients, the myeloma cells also secrete excessive amounts of light chains. These excess light chains were first discovered in the urine of myeloma patients and were named **Bence-Jones proteins,** for their discoverer.

Multiple myeloma also occurs in other animals. In mice it can arise spontaneously, as it does in humans, or conditions favoring myeloma induction can be created by injecting mineral oil into the peritoneal cavity. The clones of malignant plasma cells that develop are called **plasmacytomas,** and many of these are designated MOPCs, denoting the mineral-oil induction of plasmacytoma cells. A large number of mouse MOPC lines secreting different immunoglobulin classes are presently carried by the American Type-Culture Collection, a nonprofit repository of cell lines commonly used in research.

Light-Chain Sequencing Revealed That Immunoglobulins Have Constant and Variable Regions

When the amino acid sequences of several Bence-Jones proteins (light chains) from different individuals were compared, a striking pattern emerged. The amino-terminal half of the chain, consisting of 100–110 amino acids, was found to vary among different Bence-Jones proteins. This region was called the **variable (V) region.** The carboxyl-terminal half of the molecule, called the **constant (C) region,** had two basic amino acid sequences. This led to the recognition that there were two light chain types, **kappa (κ)** and **lambda (λ).** In humans, 60% of the light chains are kappa and 40% are lambda, whereas in mice, 95% of the light chains are kappa and only 5% are lambda. A single antibody molecule contains only one light chain type, either κ or λ, never both.

The amino acid sequences of λ light chains show minor differences that are used to classify λ light chains into subtypes. In mice, there are three subtypes (λ1, λ2, and λ3); in humans, there are four subtypes. Amino acid substitutions at only a few positions are responsible for the subtype differences.

Heavy-Chain Sequencing Revealed Five Basic Varieties of Heavy Chains

For heavy-chain sequencing studies, myeloma proteins were reduced with mercaptoethanol and alkylated, and the heavy chains were separated by gel filtration in a denaturing solvent. When the amino acid sequences of several myeloma protein heavy chains were compared, a pattern similar to that of the light chains emerged. The amino-terminal part of the chain, consisting of 100–110 amino acids, showed great sequence variation among myeloma heavy chains and was therefore called the variable (V) region. The remaining part of the protein revealed five basic sequence patterns, corresponding to five different heavy-chain constant (C) regions (μ, δ, γ, ϵ and α). Each of these five different heavy chains is called an **isotype.** The length of the constant regions is approximately 330 amino acids for δ, γ, and α, and 440 amino acids for μ and ε. The heavy chains of a given antibody molecule determine the class of that antibody: IgM(μ), IgG(γ), IgA(α), IgD(δ), or IgE(ϵ). Each class can have either κ or λ light chains. A single antibody molecule has two identical heavy chains and two identical light chains, H_2L_2, or a multiple $(H_2L_2)_n$ of this basic four-chain structure (Table 4-1).

Minor differences in the amino acid sequences of the α and γ heavy chains led to further classification of the heavy chains into subisotypes that determine the subclass of antibody molecules they constitute. In humans, there are two subisotypes of α heavy chains—α1 and α2—(and thus two subclasses, IgA1 and IgA2)—and four subisotypes of γ heavy chains: γ1, γ2, γ3, and γ4 (therefore four subclasses, IgG1, IgG2, IgG3, and IgG4). In mice, there are four subisotypes, γ1, γ2a, γ2b, and γ3, and the corresponding subclasses.

Immunoglobulin Fine Structure

The structure of the immunoglobulin molecule is determined by the primary, secondary, tertiary, and quaternary organization of the protein. The primary structure, the amino acid sequence, accounts for the variable and constant regions of the heavy and light chains. The secondary structure is formed by folding of the extended polypeptide chain

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An Introduction to Immunoglobulin Structure

TABLE 4-1 Chain composition of the five immunoglobulin classes in humans

Class	Heavy chain	Subclasses	Light chain	Molecular formula
IgG	γ	$\gamma1$, $\gamma2$, $\gamma3$, $\gamma4$	κ or λ	$\gamma_2\kappa_2$ $\gamma_2\lambda_2$
IgM	μ	None	κ or λ	$(\mu_2\kappa_2)_n$ $(\mu_2\lambda_2)_n$ $n = 1$ or 5
IgA	α	$\alpha1$, $\alpha2$	κ or λ	$(\alpha_2\kappa_2)_n$ $(\alpha_2\lambda_2)_n$ $n = 1, 2, 3$, or 4
IgE	ϵ	None	κ or λ	$\epsilon_2\kappa_2$ $\epsilon_2\lambda_2$
IgD	δ	None	κ or λ	$\delta_2\kappa_2$ $\delta_2\lambda_2$

back and forth upon itself into an antiparallel β pleated sheet (Figure 4-4). The chains are then folded into a tertiary structure of compact globular domains, which are connected to neighboring domains by continuations of the polypeptide chain that lie outside the β pleated sheets. Finally, the globular domains of adjacent heavy and light polypeptide chains interact in the quaternary structure (Figure 4-5), forming functional domains that enable the molecule to specifically bind antigen and, at the same time, perform a number of biological effector functions.

Immunoglobulins Possess Multiple Domains Based on the Immunoglobulin Fold

Careful analysis of the amino acid sequences of immunoglobulin heavy and light chains showed that both chains contain several homologous units of about 110 amino acid residues. Within each unit, termed a domain, an intrachain disulfide bond forms a loop of about 60 amino acids. Light chains contain one variable domain (V_L), and one constant domain (C_L); heavy chains contain one variable domain (V_H), and either three or four constant domains (C_H1, C_H2, C_H3, and C_H4), depending on the antibody class (Figure 4-6).

X-ray crystallographic analysis revealed that immunoglobulin domains are folded into a characteristic compact structure called the **immunoglobulin fold.** This structure consists of a "sandwich" of two β pleated sheets, each containing antiparallel β strands of amino acids, which are connected by loops of various lengths (Figure 4-7). The β strands within a sheet are stabilized by hydrogen bonds that connect the –NH groups in one strand with carbonyl groups of an adjacent strand (see Figure 4-4). The β strands are characterized by alternating hydrophobic and hydrophilic amino acids whose side chains are arranged perpendicular to the plane of the sheet; the hydrophobic amino acids are oriented toward the interior of the sandwich, and the hydrophilic amino acids face outward.

The two β sheets within an immunoglobulin fold are stabilized by the hydrophobic interactions between them and by the conserved disulfide bond. An analogy has been made to two pieces of bread, the butter between them, and a toothpick holding the slices together. The bread slices represent the two β pleated sheets; the butter represents the hydrophobic interactions between them; and the toothpick represents the intrachain disulfide bond. Although variable and constant domains have a similar structure, there are subtle differences between them. The V domain is slightly longer than the C domain and contains an extra pair of β strands within the β-sheet structure, as well as the extra loop sequence connecting this pair of β strands (see Figure 4-7).

The basic structure of the immunoglobulin fold contributes to the quaternary structure of immunoglobulins by facilitating noncovalent interactions between domains

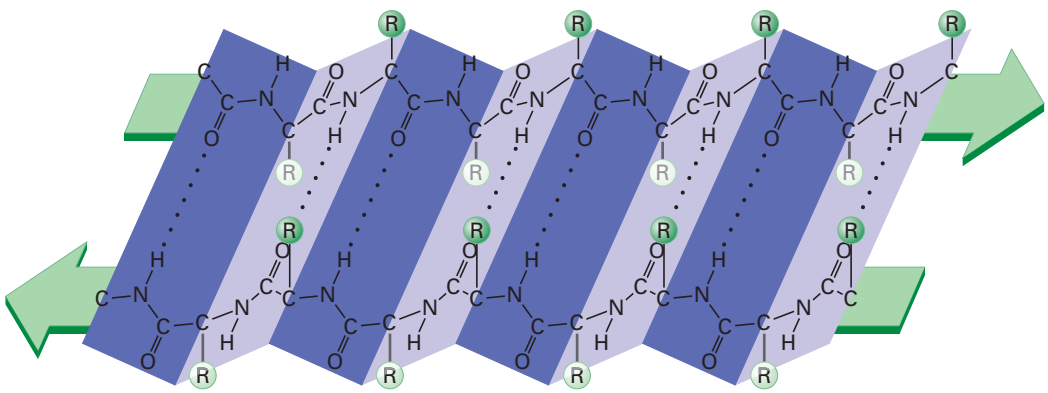

FIGURE 4-4 Structural formula of a β pleated sheet containing two antiparallel β strands. The structure is held together by hydrogen bonds between peptide bonds of neighboring stretches of polypeptide chains. The amino acid side groups (R) are arranged perpendicular to the plane of the sheet. *[Adapted from H. Lodish et al., 1995,* Molecular Cell Biology, *4th ed., Scientific American Books, New York; reprinted by permission of W. H. Freeman and Company.]*

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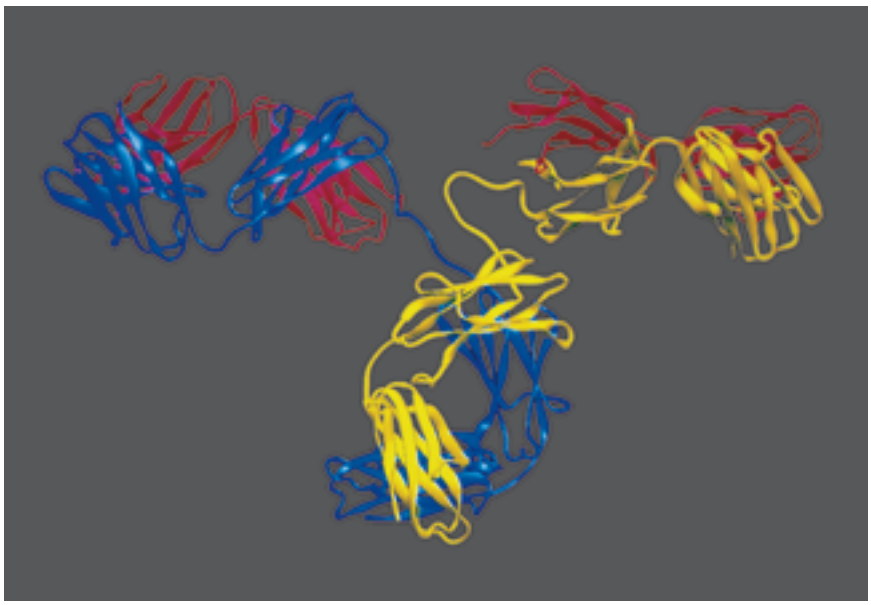

FIGURE 4-5 Ribbon representation of an intact monoclonal antibody depicting the heavy chains (yellow and blue) and light chains (red). The domains of the molecule composed of β pleated sheets are readily visible as is the extended conformation of the hinge region. *[The laboratory of A. McPherson provided this image, which is based on x-ray crystallography data determined by L. J. Harris et al., 1992,* Nature ***360:****369. The image was generated using the computer program RIBBONS.]*

across the faces of the β sheets (Figure 4-8). Interactions form links between identical domains (e.g., C_H2/C_H2, C_H3/C_H3, and C_H4/C_H4) and between nonidentical domains (e.g., V_H/V_L and C_H1/C_L). The structure of the immunoglobulin fold also allows for variable lengths and sequences of amino acids that form the loops connecting the β strands. As the next section explains, some of the loop sequences of the V_H and V_L domains contain variable amino acids and constitute the antigen-binding site of the molecule.

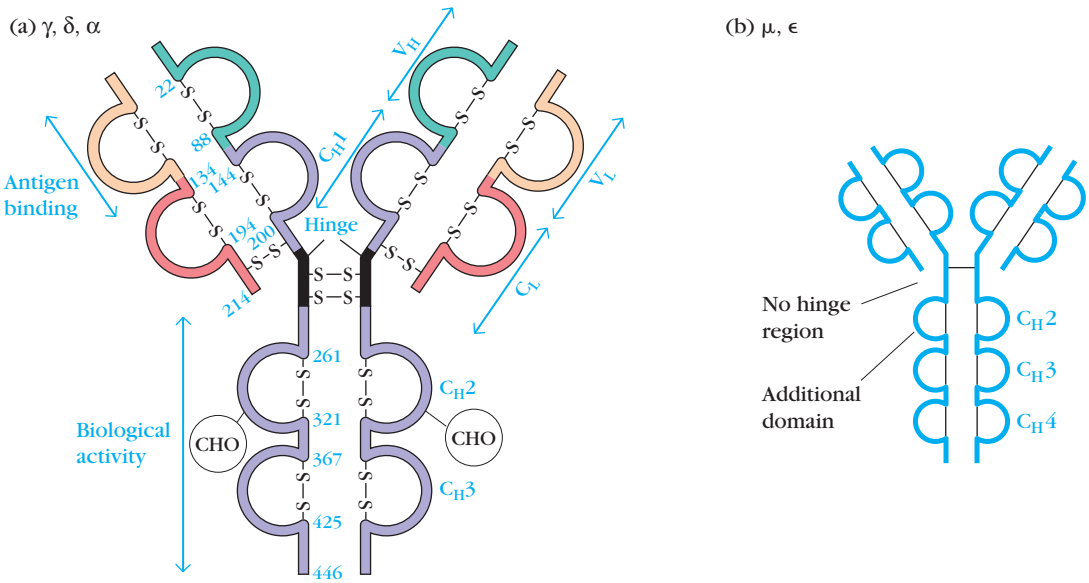

FIGURE 4-6 (a) Heavy and light chains are folded into domains, each containing about 110 amino acid residues and an intrachain disulfide bond that forms a loop of 60 amino acids. The amino-terminal domains, corresponding to the V regions, bind to antigen; effector functions are mediated by the other domains. (b) The μ and ε heavy chains contain an additional domain that replaces the hinge region.

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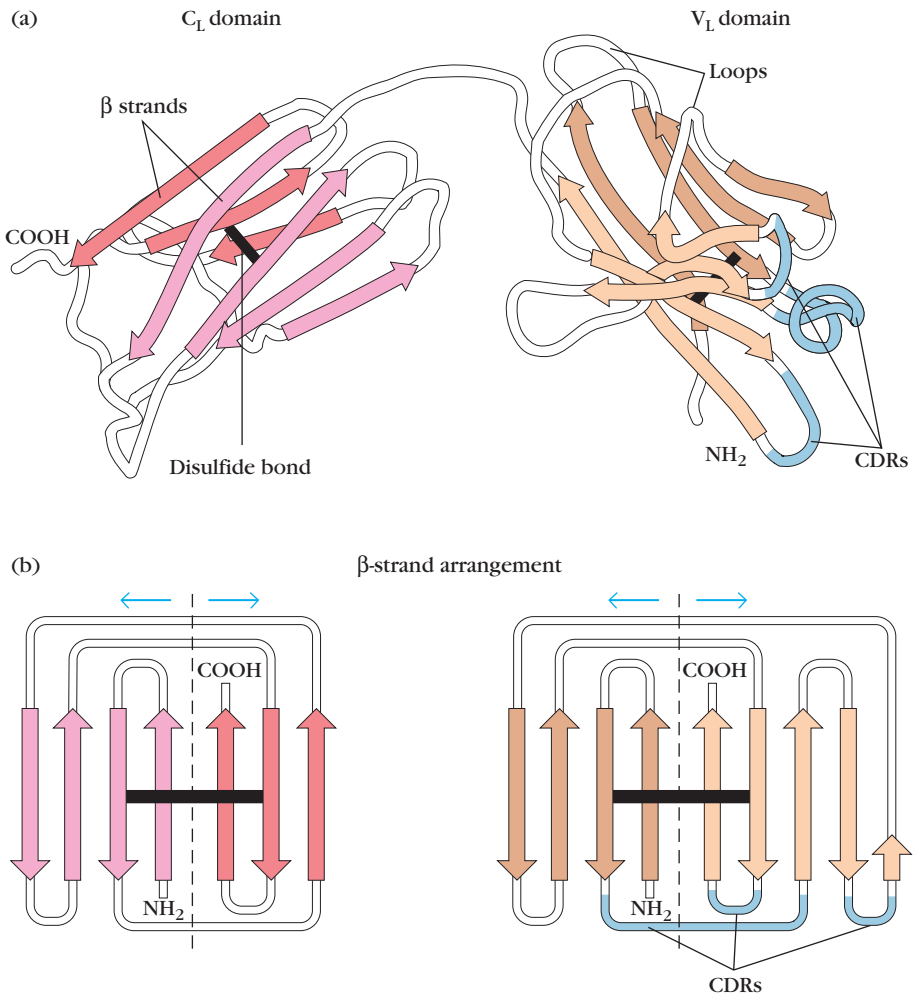

FIGURE 4-7 (a) Diagram of an immunoglobulin light chain depicting the immunoglobulin-fold structure of its variable and constant domains. The two β pleated sheets in each domain are held together by hydrophobic interactions and the conserved disulfide bond. The β strands that compose each sheet are shown in different colors. The amino acid sequences in three loops of each variable domain show considerable variation; these hypervariable regions (blue) make up the antigen-binding site. Hypervariable regions are usually called CDRs (complementarity-determining regions). Heavy-chain domains have the same characteristic structure. (b) The β pleated sheets are opened out to reveal the relationship of the individual β strands and joining loops. Note that the variable domain contains two more β strands than the constant domain. *[Part (a) adapted from M. Schiffer et al., 1973,* Biochemistry ***12:****4620; reprinted with permission; part (b) adapted from Williams and Barclay, 1988,* Annu. Rev. Immunol. ***6:****381.]*

Diversity in the Variable-Region Domain Is Concentrated in CDRs

Detailed comparisons of the amino acid sequences of a large number of V_L and V_H domains revealed that the sequence variation is concentrated in a few discrete regions of these domains. The pattern of this variation is best summarized by a quantitative plot of the variability at each position of the polypeptide chain. The **variability** is defined as:

$$\text{Variability} = \frac{\text{\# of different amino acids at a given position}}{\text{Frequency of the most common amino acid at given position}}$$

Thus if a comparison of the sequences of 100 heavy chains revealed that a serine was found in position 7 in 51 of the sequences (frequency 0.51), it would be the most common amino acid. If examination of the other 49 sequences showed that position 7 was occupied by either glutamine, histidine, proline, or tryptophan, the variability at that position would be 9.8 (5/0.51). Variability plots of V_L and V_H domains of human antibodies show that maximum variation is seen in those portions of the sequence that correspond to the loops that join the β strands (Figure 4-9). These regions were originally called **hypervariable regions** in recognition of their high variability. Hypervariable regions form the antigen-binding site of the antibody molecule. Because the antigen binding site is complementary to the structure of the epitope,

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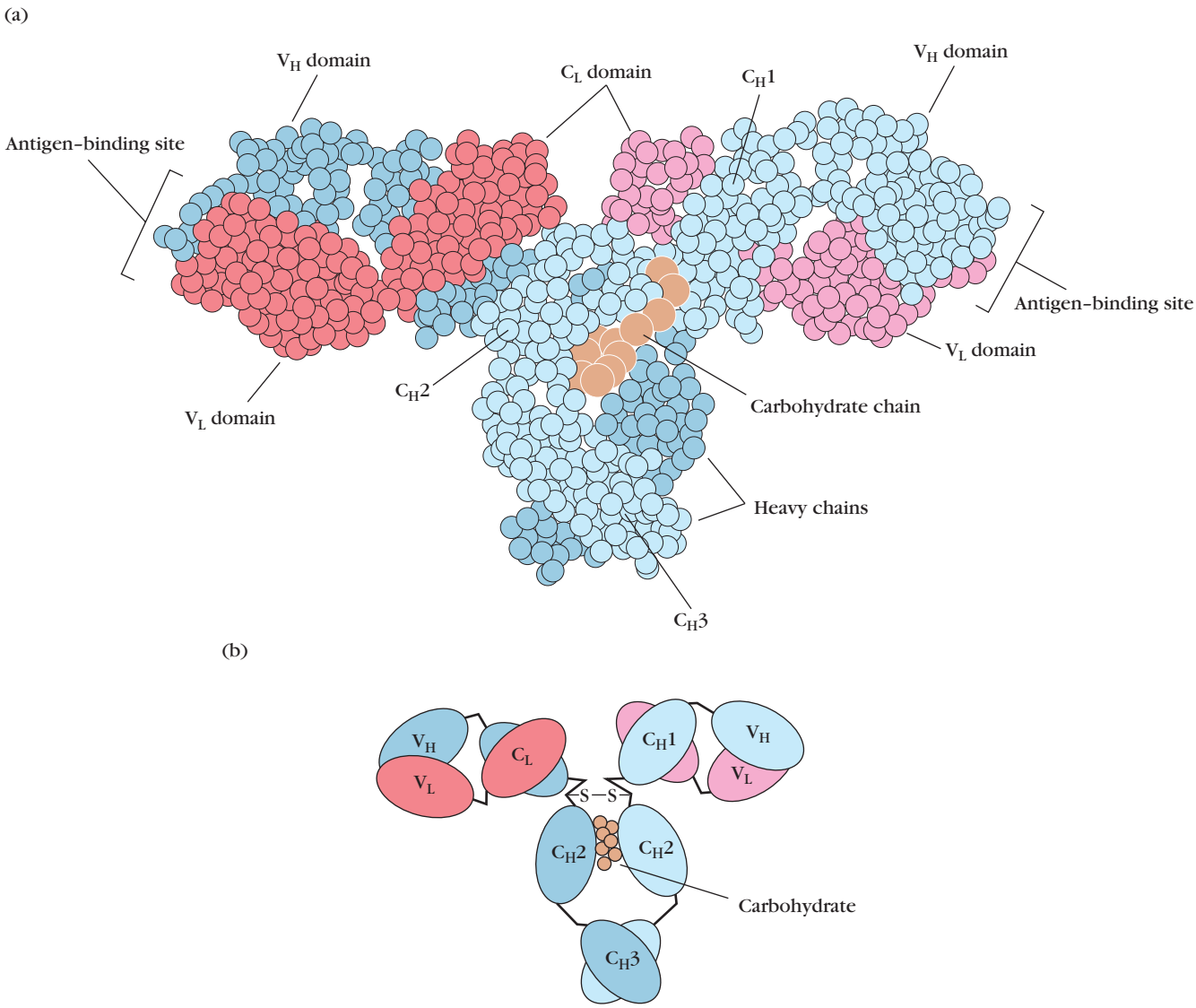

FIGURE 4-8 Interactions between domains in the separate chains of an immunoglobulin molecule are critical to its quaternary structure. (a) Model of IgG molecule, based on x-ray crystallographic analysis, showing associations between domains. Each solid ball represents an amino acid residue; the larger tan balls are carbohydrate. The two light chains are shown in shades of red; the two heavy chains, in shades of blue. (b) A schematic diagram showing the interacting heavy- and light-chain domains. Note that the C_H2/C_H2 domains protrude because of the presence of carbohydrate (tan) in the interior. The protrusion makes this domain more accessible, enabling it to interact with molecules such as certain complement components. *[Part (a) from E. W. Silverton et al., 1977,* Proc. Nat. Acad. Sci. U.S.A. ***74:****5140.]*

these areas are now more widely called **complementarity determining regions (CDRs).** The three heavy-chain and three light-chain CDR regions are located on the loops that connect the β strands of the V_H and V_L domains. The remainder of the V_L and V_H domains exhibit far less variation; these stretches are called the **framework regions (FRs).** The wide range of specificities exhibited by antibodies is due to variations in the length and amino acid sequence of the six CDRs in each Fab fragment. The framework region acts as a scaffold that supports these six loops. The three-dimensional structure of the framework regions of virtually all antibodies analyzed to date can be superimposed on one another; in contrast, the hypervariable loops (i.e., the CDRs) have different orientations in different antibodies.

CDRs Bind Antigen

The finding that CDRs are the antigen-binding regions of antibodies has been confirmed directly by high-resolution x-ray crystallography of antigen-antibody complexes. Crystallographic analysis has been completed for many Fab fragments of monoclonal antibodies complexed either with

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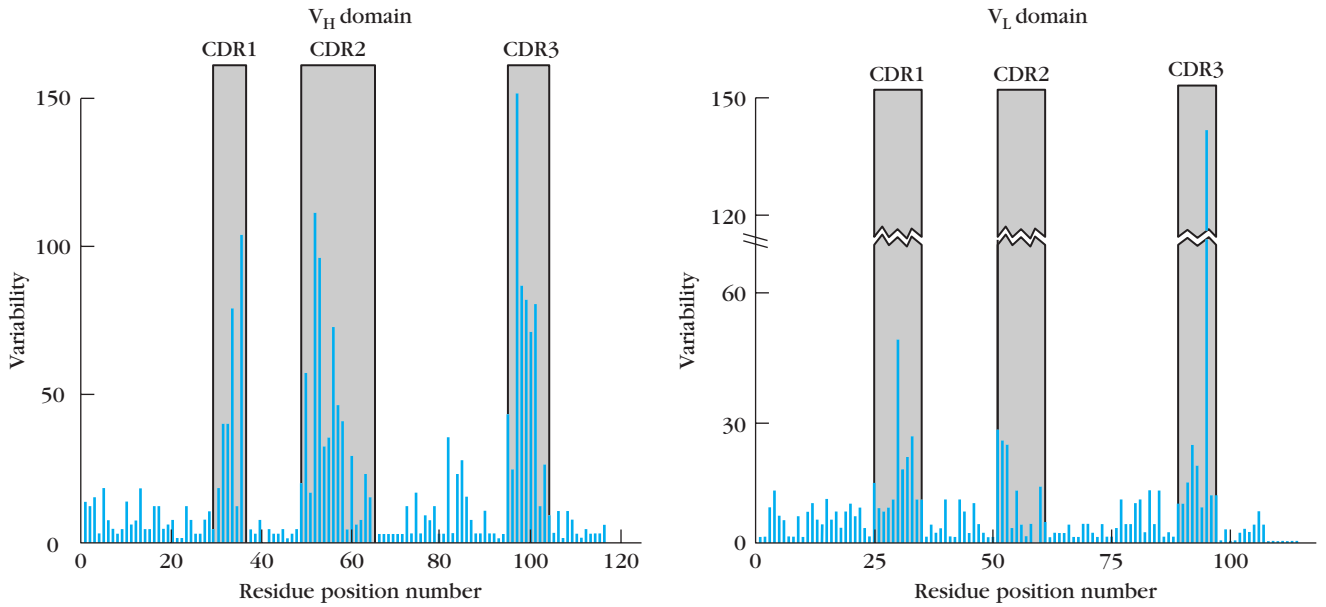

FIGURE 4-9 Variability of amino acid residues in the V_L and V_H domains of human antibodies with different specificities. Three hypervariable (HV) regions, also called complementarity-determining regions (CDRs), are present in both heavy- and light-chain V domains. As shown in Figure 4-7 (*right*), the three HV regions in the light-chain V domain are brought into proximity in the folded structure. The same is true of the heavy-chain V domain. *[Based on E. A. Kabat et al., 1977,* Sequence of Immunoglobulin Chains, *U.S. Dept. of Health, Education, and Welfare.]*

large globular protein antigens or with a number of smaller antigens including carbohydrates, nucleic acids, peptides, and small haptens. In addition, complete structures have been obtained for several intact monoclonal antibodies. X-ray diffraction analysis of antibody-antigen complexes has shown that several CDRs may make contact with the antigen, and a number of complexes have been observed in which all six CDRs contact the antigen. In general, more residues in the heavy-chain CDRs appear to contact antigen than in the light-chain CDRs. Thus the V_H domain often contributes

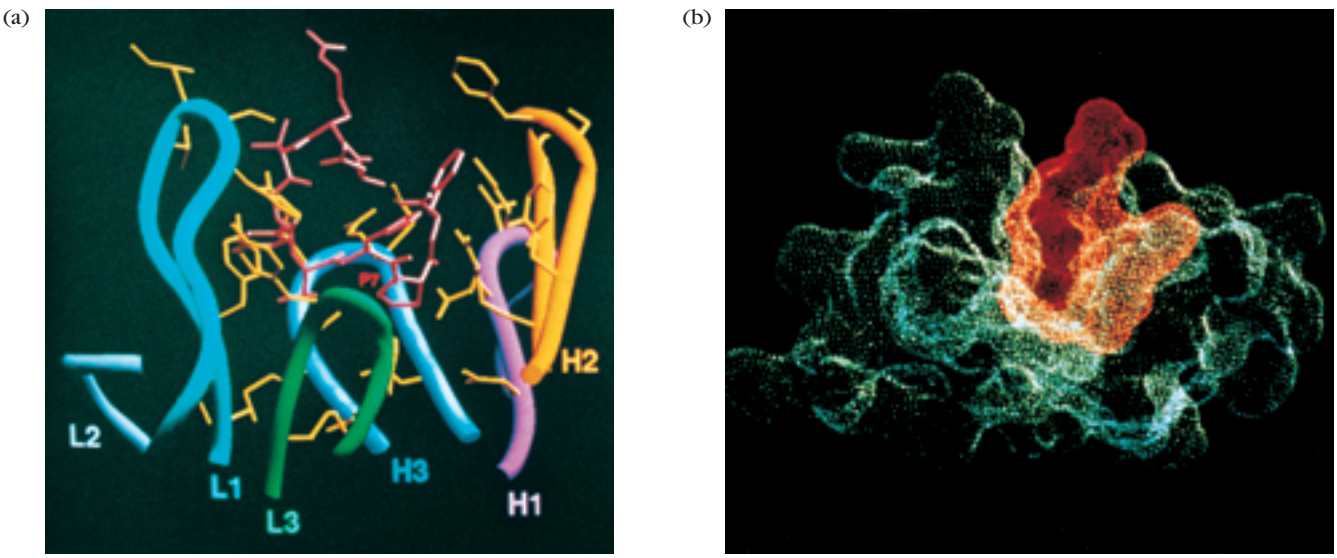

FIGURE 4-10 (a) Side view of the three-dimensional structure of the combining site of an angiotensin II–Fab complex. The peptide is in red. The three heavy-chain CDRs (H1, H2, H3) and three light-chain CDRs (L1, L2, L3) are each shown in a different color. All six CDRs contain side chains, shown in yellow, that are within van der Waals contact of the angiotensin peptide. (b) Side view of the van der Waals surface of contact between angiotensin II and Fab fragment. *[From K. C. Garcia et al., 1992,* Science ***257:****502; courtesy of M. Amzel, Johns Hopkins University.]*

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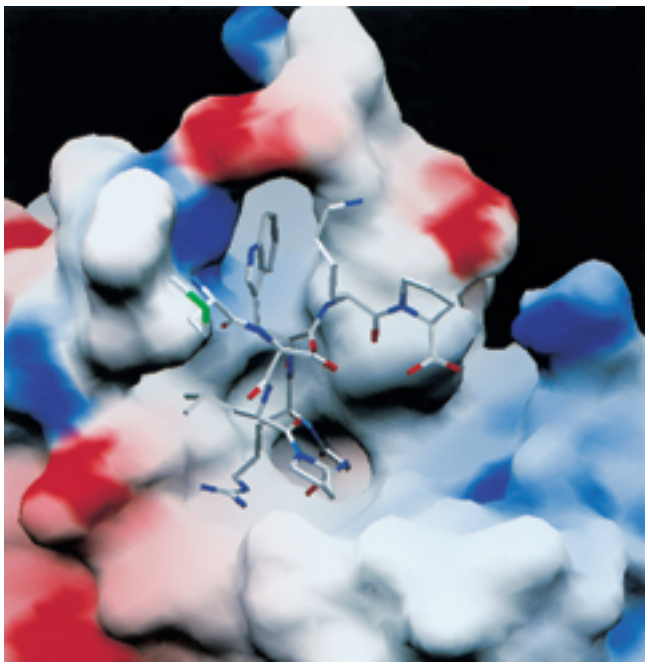
more to antigen binding than the V_L domain. The dominant role of the heavy chain in antigen binding was demonstrated in a study in which a single heavy chain specific for a glycoprotein antigen of the human immunodeficiency virus (HIV) was combined with various light chains of different antigenic specificity. All of the hybrid antibodies bound to the HIV glycoprotein antigen, indicating that the heavy chain alone was sufficient to confer specificity. However, one should not conclude that the light chain is largely irrelevant; in some antibody-antigen reactions, the light chain makes the more important contribution.

The actual shape of the antigen binding site formed by whatever combination of CDRs are used in a particular antibody has been shown to vary dramatically. As pointed out in Chapter 3, contacts between a large globular protein antigen and antibody occur over a broad, often rather flat, undulating face. In the area of contact, protrusions or depressions on the antigen are likely to match complementary depressions or protrusions on the antibody. In the case of the well studied lysozyme/anti-lysozyme system, crystallographic studies have shown that the surface areas of interaction are quite large, ranging from about 650 $Å^2$ to more than 900 $Å^2$. Within this area, some 15–22 amino acids in the antibody contact the same number of residues in the protein antigen. In contrast, antibodies bind smaller antigens, such as small haptens, in smaller, recessed pockets in which the ligand is buried. This is nicely illustrated by the interaction of the small octapeptide hormone angiotensin II with the binding site of an anti-angiotensin antibody (Figure 4-10).

Conformational Changes May Be Induced by Antigen Binding

As more x-ray crystallographic analyses of Fab fragments were completed, it became clear that in some cases binding of antigen induces conformational changes in the antibody, antigen, or both. Formation of the complex between neuraminidase and anti-neuraminidase is accompanied by a change in the orientation of side chains of both the epitope and the antigen-binding site. This conformational change results in a closer fit between the epitope and the antibody's binding site.

In another example, comparison of an anti-hemagglutinin Fab fragment before and after binding to a hemagglutinin peptide antigen has revealed a visible conformational change in the heavy-chain CDR3 loop and in the accessible surface of the binding site. Another striking example of conformational change has been seen in the complex between an Fab fragment derived from a monoclonal antibody against the HIV protease and the peptide epitope of the protease. As shown in Figure 4-11, there are significant changes in the Fab upon binding. In fact, upon antigen binding, the CDR1 region of the light chain moves as much as 1 Å and the heavy chain CDR3 moves 2.7 Å. Thus, in addition to variability in the



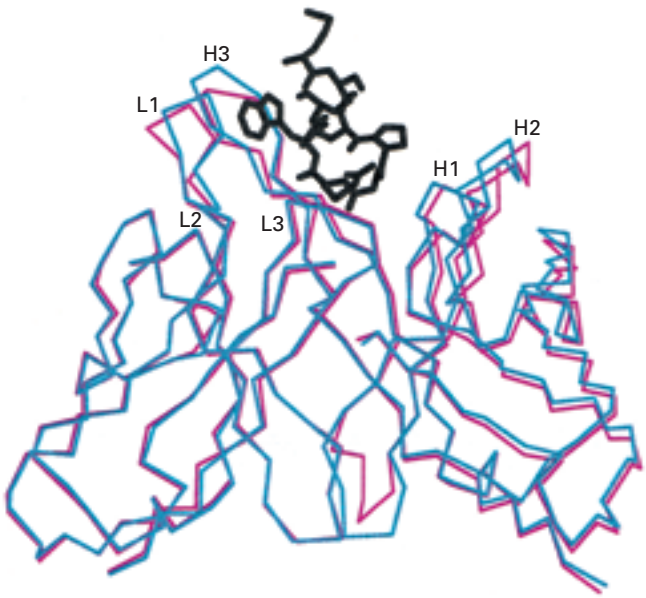


FIGURE 4-11 Structure of a complex between a peptide derived from HIV protease and an Fab fragment from an anti-protease antibody (*left*) and comparison of the Fab structure before and after peptide binding (*right*). In the right panel, the red line shows the structure of the Fab fragment before it binds the peptide and the blue line shows its structure when bound. There are significant conformational changes in the CDRs of the Fab on binding the antigen. These are especially pronounced in the light chain CDR1 (L1) and the heavy chain CDR3 (H3). *[From J. Lescar et al., 1997,* J. Mol. Biol. ***267:****1207; courtesy of G. Bentley, Institute Pasteur.]*

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length and amino acid composition of the CDR loops, the ability of these loops to significantly change conformation upon antigen binding enables antibodies to assume a shape more effectively complementary to that of their epitopes.

As already indicated, conformational changes following antigen binding need not be limited to the antibody. Although it is not shown in Figure 4-11, the conformation of the protease peptide bound to the Fab shows no structural similarity to the corresponding epitope in the native HIV protease. It has been suggested that the inhibition of protease activity by this anti-HIV protease antibody is a result of its distortion of the enzyme's native conformation.

Constant-Region Domains

The immunoglobulin constant-region domains take part in various biological functions that are determined by the amino acid sequence of each domain.

C_{H1} AND C_L DOMAINS

The C_H1 and C_L domains serve to extend the Fab arms of the antibody molecule, thereby facilitating interaction with antigen and increasing the maximum rotation of the Fab arms. In addition, these constant-region domains help to hold the V_H and V_L domains together by virtue of the interchain disulfide bond between them (see Figure 4-6). Also, the C_H1 and C_L domains may contribute to antibody diversity by allowing more random associations between V_H and V_L domains than would occur if this association were driven by the V_H/V_L interaction alone. These considerations have important implications for building a diverse repertoire of antibodies. As Chapter 5 will show, random rearrangements of the immunoglobulin genes generate unique V_H and V_L sequences for the heavy and light chains expressed by each B lymphocyte; association of the V_H and V_L sequences then generates a unique antigen-binding site. The presence of C_H1 and C_L domains appears to increase the number of stable V_H and V_L interactions that are possible, thus contributing to the overall diversity of antibody molecules that can be expressed by an animal.

HINGE REGION

The γ, δ, and α heavy chains contain an extended peptide sequence between the C_H1 and C_H2 domains that has no homology with the other domains (see Figure 4-8). This region, called the **hinge region,** is rich in proline residues and is flexible, giving IgG, IgD, and IgA segmental flexibility. As a result, the two Fab arms can assume various angles to each other when antigen is bound. This flexibility of the hinge region can be visualized in electron micrographs of antigen-antibody complexes. For example, when a molecule containing two dinitrophenol (DNP) groups reacts with anti-DNP antibody and the complex is captured on a grid, negatively stained, and observed by electron microscopy, large complexes (e.g., dimers, trimers, tetramers) are seen. The angle between the arms of the Y-shaped antibody molecules differs in the different complexes, reflecting the flexibility of the hinge region (Figure 4-12).

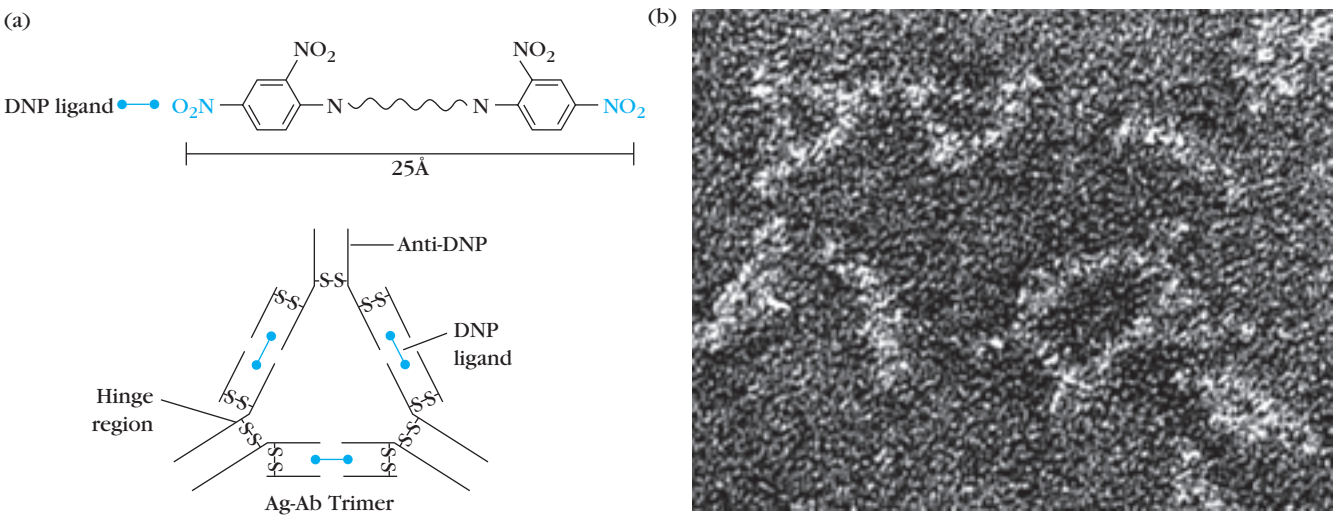

FIGURE 4-12 Experimental demonstration of the flexibility of the hinge region in antibody molecules. (a) A hapten in which two dinitrophenyl (DNP) groups are tethered by a short connecting spacer group reacts with anti-DNP antibodies to form trimers, tetramers, and other larger antigen-antibody complexes. A trimer is shown schematically. (b) In an electron micrograph of a negatively stained preparation of these complexes, two triangular trimeric structures are clearly visible. The antibody protein stands out as a light structure against the electron-dense background. Because of the flexibility of the hinge region, the angle between the arms of the antibody molecules varies. *[Photograph from R. C. Valentine and N. M. Green, 1967,* J. Mol. Biol. ***27:****615; reprinted by permission of Academic Press Inc. (London) Ltd.]*

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Two prominent amino acids in the hinge region are proline and cysteine. The large number of proline residues in the hinge region gives it an extended polypeptide conformation, making it particularly vulnerable to cleavage by proteolytic enzymes; it is this region that is cleaved with papain or pepsin (see Figure 4-3). The cysteine residues form interchain disulfide bonds that hold the two heavy chains together. The number of interchain disulfide bonds in the hinge region varies considerably among different classes of antibodies and between species. Although μ and ϵ chains lack a hinge region, they have an additional domain of 110 amino acids (C_H2/C_H2) that has hingelike features.

OTHER CONSTANT-REGION DOMAINS

As noted already, the heavy chains in IgA, IgD, and IgG contain three constant-region domains and a hinge region, whereas the heavy chains in IgE and IgM contain four constant-region domains and no hinge region. The corresponding domains of the two groups are as follows:

IgA, IgD, IgG	IgE, IgM
C_H1/C_H1	C_H1/C_H1
Hinge region	C_H2/C_H2
C_H2/C_H2	C_H3/C_H3
C_H3/C_H3	C_H4/C_H4

Although the C_H2/C_H2 domains in IgE and IgM occupy the same position in the polypeptide chains as the hinge region in the other classes of immunoglobulin, a function for this extra domain has not yet been determined.

X-ray crystallographic analyses have revealed that the two C_H2 domains of IgA, IgD, and IgG (and the C_H3 domains of IgE and IgM) are separated by oligosaccharide side chains; as a result, these two globular domains are much more accessible than the others to the aqueous environment (see Figure 4-8b). This accessibility is one of the elements that contributes to the biological activity of these domains in the activation of complement components by IgG and IgM.

The carboxyl-terminal domain is designated C_H3/C_H3 in IgA, IgD, and IgG and C_H4/C_H4 in IgE and IgM. The five classes of antibody and their subclasses can be expressed either as **secreted immunoglobulin (sIg)** or as **membrane-bound immunoglobulin (mIg).** The carboxyl-terminal domain in secreted immunoglobulin differs in both structure and function from the corresponding domain in membrane-bound immunoglobulin. Secreted immunoglobulin has a hydrophilic amino acid sequence of various lengths at the carboxyl-terminal end. The functions of this domain in the various classes of secreted antibody will be described later. In membrane-bound immunoglobulin, the carboxyl-terminal domain contains three regions:

- An extracellular hydrophilic "spacer" sequence composed of 26 amino acid residues
- A hydrophobic transmembrane sequence
- A short cytoplasmic tail

The length of the transmembrane sequence is constant among all immunoglobulin isotypes, whereas the lengths of the extracellular spacer sequence and the cytoplasmic tail vary.

B cells express different classes of mIg at different developmental stages. The immature B cell, called a pre-B cell, expresses only mIgM; later in maturation, mIgD appears and is coexpressed with IgM on the surface of mature B cells before they have been activated by antigen. A memory B cell can express mIgM, mIgG, mIgA, or mIgE. Even when different classes are expressed sequentially on a single cell, the antigenic specificity of all the membrane antibody molecules expressed by a single cell is identical, so that each antibody molecule binds to the same epitope. The genetic mechanism that allows a single B cell to express multiple immunoglobulin isotypes all with the same antigenic specificity is described in Chapter 5.

Antibody-Mediated Effector Functions

In addition to binding antigen, antibodies participate in a broad range of other biological activities. When considering the role of antibody in defending against disease, it is important to remember that antibodies generally do not kill or remove pathogens solely by binding to them. In order to be effective against pathogens, antibodies must not only recognize antigen, but also invoke responses—effector functions—that will result in removal of the antigen and death of the pathogen. While variable regions of antibody are the sole agents of binding to antigen, the heavy-chain constant region (C_H) is responsible for a variety of collaborative interactions with other proteins, cells, and tissues that result in the effector functions of the humoral response.

Because these effector functions result from interactions between heavy-chain constant regions and other serum proteins or cell-membrane receptors, not all classes of immunoglobulin have the same functional properties. An overview of four major effector functions mediated by domains of the constant region is presented here. A fifth function unique to IgE, the activation of mast cells, eosinophils, and basophils, will be described later.

Opsonization Is Promoted by Antibody

Opsonization, the promotion of phagocytosis of antigens by macrophages and neutrophils, is an important factor in antibacterial defenses. Protein molecules called *Fc receptors (FcR),* which can bind the constant region of Ig molecules, are present on the surfaces of macrophages and neutrophils.

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The binding of phagocyte Fc receptors with several antibody molecules complexed with the same target, such as a bacterial cell, produces an interaction that results in the binding of the pathogen to the phagocyte membrane. This crosslinking of the FcR by binding to an array of antibody Fc regions initiates a signal-transduction pathway that results in the phagocytosis of the antigen-antibody complex. Inside the phagocyte, the pathogen becomes the target of various destructive processes that include enzymatic digestion, oxidative damage, and the membrane-disrupting effects of antibacterial peptides.

Antibodies Activate Complement

IgM and, in humans, most IgG subclasses can activate a collection of serum glycoproteins called the **complement system.** Complement includes a collection of proteins that can perforate cell membranes. An important byproduct of the complement activation pathway is a protein fragment called C3b, which binds nonspecifically to cell- and antigen-antibody complexes near the site at which complement was activated. Many cell types—for example, red blood cells and macrophages—have receptors for C3b and so bind cells or complexes to which C3b has adhered. Binding of adherent C3b by macrophages leads to phagocytosis of the cells or molecular complexes attached to C3b. Binding of antigen-antibody complexes by the C3b receptors of a red blood cell allows the erythrocyte to deliver the complexes to liver or spleen, where resident macrophages remove them without destroying the red cell. The collaboration between antibody and the complement system is important for the inactivation and removal of antigens and the killing of pathogens. The process of complement activation is described in detail in Chapter 13.

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) Kills Cells

The linking of antibody bound to target cells (virus infected cells of the host) with the Fc receptors of a number of cell types, particularly natural killer (NK) cells, can direct the cytotoxic activities of the effector cell against the target cell. In this process, called **antibody-dependent cell-mediated cytotoxicity (ADCC),** the antibody acts as a newly acquired receptor enabling the attacking cell to recognize and kill the target cell. The phenomenon of ADCC is discussed in Chapter 14.

Some Antibodies Can Cross Epithelial Layers by Transcytosis

The delivery of antibody to the mucosal surfaces of the respiratory, gastrointestinal, and urogenital tracts, as well as its export to breast milk, requires the movement of immunoglobulin across epithelial layers, a process called **transcytosis.** The capacity to be transported depends on properties of the constant region. In humans and mice, IgA is the major antibody species that undergoes such transcytosis, although IgM can also be transported to mucosal surfaces. Some mammalian species, such as humans and mice, also transfer significant amounts of most subclasses of IgG from mother to fetus. Since maternal and fetal circulatory systems are separate, antibody must be transported across the placental tissue that separates mother and fetus. In humans, this transfer takes place during the third trimester of gestation. The important consequence is that the developing fetus receives a sample of the mother's repertoire of antibody as a protective endowment against pathogens. As with the other effector functions described here, the capacity to undergo transplacental transport depends upon properties of the constant region of the antibody molecule.

The transfer of IgG from mother to fetus is a form of **passive immunization,** which is the acquisition of immunity by receipt of preformed antibodies rather than by active production of antibodies after exposure to antigen. The ability to transfer immunity from one individual to another by the transfer of antibodies is the basis of passive antibody therapy, an important and widely practiced medical procedure (see Clinical Focus).

Antibody Classes and Biological Activities

The various immunoglobulin isotypes and classes have been mentioned briefly already. Each class is distinguished by unique amino acid sequences in the heavy-chain constant region that confer class-specific structural and functional properties. In this section, the structure and effector functions of each class are described in more detail. The molecular properties and biological activities of the immunoglobulin classes are summarized in Table 4-2 (page 90). The structures of the five major classes are diagramed in Figure 4-13 (page 91).

Immunoglobulin G (IgG)

IgG, the most abundant class in serum, constitutes about 80% of the total serum immunoglobulin. The IgG molecule consists of two γ heavy chains and two κ or two λ light chains (see Figure 4-13a). There are four human IgG subclasses, distinguished by differences in γ-chain sequence and numbered according to their decreasing average serum concentrations: IgG1, IgG2, IgG3, and IgG4 (see Table 4-2).

The amino acid sequences that distinguish the four IgG subclasses are encoded by different germ-line C_H genes, whose DNA sequences are 90%–95% homologous. The structural characteristics that distinguish these subclasses from one another are the size of the hinge region and the number and position of the interchain disulfide bonds between the heavy chains (Figure 4-14, page 92). The subtle

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CLINICAL FOCUS

Passive Antibody Therapy

In 1890, Emil Behring and Shibasaburo Kitasato reported an extraordinary experiment. They immunized rabbits with tetanus and then collected serum from these animals. Subsequently, they injected 0.2 ml of the immune serum into the abdominal cavity of six mice. After 24 hours, they infected the treated animals and untreated controls with live, virulent tetanus bacteria. All of the control mice died within 48 hours of infection, whereas the treated mice not only survived but showed no effects of infection. This landmark experiment demonstrated two important points. One, it showed that following immunization, substances appeared in serum that could protect an animal against pathogens. Two, this work demonstrated that immunity could be passively acquired. Immunity could be transferred from one animal to another by taking serum from an immune animal and injecting it into a nonimmune one. These and subsequent experiments did not go unnoticed. Both men eventually received titles (Behring became von Behring and Kitasato became Baron Kitasato). A few years later, in 1901, von Behring was awarded the first Nobel prize in Medicine.

These early observations and others paved the way for the introduction of passive immunization into clinical practice. During the 1930s and 1940s, passive immunotherapy, the endowment of resistance to pathogens by transfer of the agent of immunity from an immunized donor to an unimmunized recipient, was used to prevent or modify the course of measles and hepatitis A. During subsequent years, clinical experience and advances in the technology of preparation of immunoglobulin for passive immunization have made this approach a standard medical practice. Passive immunization based on the transfer of antibodies is widely used in the treatment of immunodeficiency diseases and as a protection against anticipated exposure to infectious agents against which one does not have immunity.

Immunoglobulin for passive immunization is prepared from the pooled plasma of thousands of donors. In effect, recipients of these antibody preparations are receiving a sample of the antibodies produced by many people to a broad diversity of different pathogens. In fact a gram of intravenous immune globulin (IVIG) contains about 10^{18} molecules of antibody (mostly IgG) and may incorporate more than 10^{7} different antibody specificities. During the course of therapy, patients receive significant amounts of IVIG, usually 200–400 mg per kilogram of body weight. This means that an immunodeficient patient weighing 70 kilograms would receive 14 to 28 grams of IVIG every 3 to 4 weeks. A product derived from the blood of such a large number of donors carries a risk of harboring pathogenic agents, particularly viruses. The risk is minimized by the processes used to produce intravenous immune globulin. The manufacture of IVIG involves treatment with solvents, such as ethanol, and the use of detergents that are highly effective in inactivating viruses such as HIV and hepatitis. In addition to removing or inactivating infectious agents, the production process must also eliminate aggregated immunoglobulin, because antibody aggregates can trigger massive activation of the complement pathway, leading to severe, even fatal, anaphylaxis.

Passively administered antibody exerts its protective action in a number of ways. One of the most important is the recruitment of the complement pathway to the destruction or removal of a pathogen. In bacterial infections, antibody binding to bacterial surfaces promotes opsonization, the phagocytosis and killing of the invader by macrophages and neutrophils. Toxins and viruses can be bound and neutralized by antibody, even as the antibody marks the pathogen for removal from the body by phagocytes and by organs such as liver and kidneys. By the initiation of antibody-dependent cell-mediated cytotoxicity (ADCC), antibodies can also mediate the killing of target cells by cytotoxic cell populations such as natural killer cells.

amino acid differences between subclasses of IgG affect the biological activity of the molecule:

- IgG1, IgG3, and IgG4 readily cross the placenta and play an important role in protecting the developing fetus.
- IgG3 is the most effective complement activator, followed by IgG1; IgG2 is less efficient, and IgG4 is not able to activate complement at all.
- IgG1 and IgG3 bind with high affinity to Fc receptors on phagocytic cells and thus mediate opsonization. IgG4 has an intermediate affinity for Fc receptors, and IgG2 has an extremely low affinity.

Immunoglobulin M (IgM)

IgM accounts for 5%–10% of the total serum immunoglobulin, with an average serum concentration of 1.5 mg/ml. Monomeric IgM, with a molecular weight of 180,000, is expressed as membrane-bound antibody on B cells. IgM is secreted by plasma cells as a pentamer in which five monomer units are held together by disulfide bonds that link their carboxyl-terminal heavy chain domains ($C_\mu4/C_\mu4$) and their $C_\mu3/C_\mu3$ domains (see Figure 4-13e). The five monomer subunits are arranged with their Fc regions in the center of the pentamer and the ten antigen-binding sites on the periphery of the molecule. Each pentamer contains an

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TABLE 4-2 Properties and biological activities* of classes and subclasses of human serum immunoglobulins

Property/Activity	IgG1	IgG2	IgG3	IgG4	IgA1	IgA2	IgM‡	IgE	IgD
Molecular weight†	150,000	150,000	150,000	150,000	150,000–600,000	150,000–600,000	900,000	190,000	150,000
Heavy-chain component	γ1	γ2	γ3	γ4	α1	α2	μ	ε	δ
Normal serum level (mg/ml)	9	3	1	0.5	3.0	0.5	1.5	0.0003	0.03
In vivo serum half life (days)	23	23	8	23	6	6	5	2.5	3
Activates classical complement pathway	+	+/−	++	−	−	−	+++	−	−
Crosses placenta	+	+/−	+	+	−	−	−	−	−
Present on membrane of mature B cells	−	−	−	−	−	−	+	−	+
Binds to Fc receptors of phagocytes	++	+/−	++	+	−	−	?	−	−
Mucosal transport	−	−	−	−	++	++	+	−	−
Induces mast-cell degranulation	−	−	−	−	−	−	−	+	−

*Activity levels indicated as follows: ++ = high; + = moderate; +/− = minimal; − = none; ? = questionable.

†IgG, IgE, and IgD always exist as monomers; IgA can exist as a monomer, dimer, trimer, or tetramer. Membrane-bound IgM is a monomer, but secreted IgM in serum is a pentamer.

‡IgM is the first isotype produced by the neonate and during a primary immune response.

additional Fc-linked polypeptide called the **J (joining) chain,** which is disulfide-bonded to the carboxyl-terminal cysteine residue of two of the ten μ chains. The J chain appears to be required for polymerization of the monomers to form pentameric IgM; it is added just before secretion of the pentamer.

IgM is the first immunoglobulin class produced in a primary response to an antigen, and it is also the first immunoglobulin to be synthesized by the neonate. Because of its pentameric structure with 10 antigen-binding sites, serum IgM has a higher valency than the other isotypes. An IgM molecule can bind 10 small hapten molecules; however, because of steric hindrance, only 5 or fewer molecules of larger antigens can be bound simultaneously. Because of its high valency, pentameric IgM is more efficient than other isotypes in binding antigens with many repeating epitopes such as viral particles and red blood cells (RBCs). For example, when RBCs are incubated with specific antibody, they clump together into large aggregates in a process called agglutination. It takes 100 to 1000 times more molecules of IgG than of IgM to achieve the same level of agglutination. A similar phenomenon occurs with viral particles: less IgM than IgG is required to neutralize viral infectivity. IgM is also more efficient than IgG at activating complement. Complement activation requires two Fc regions in close proximity, and the pentameric structure of a single molecule of IgM fulfills this requirement.

Because of its large size, IgM does not diffuse well and therefore is found in very low concentrations in the intercellular tissue fluids. The presence of the J chain allows IgM to bind to receptors on secretory cells, which transport it across epithelial linings to enter the external secretions that bathe mucosal surfaces. Although IgA is the major isotype found in these secretions, IgM plays an important accessory role as a secretory immunoglobulin.

Immunoglobulin A (IgA)

Although IgA constitutes only 10%–15% of the total immunoglobulin in serum, it is the predominant immunoglobulin class in external secretions such as breast milk, saliva, tears, and mucus of the bronchial, genitourinary, and digestive tracts. In serum, IgA exists primarily as a monomer, but polymeric forms (dimers, trimers, and some tetramers) are sometimes seen, all containing a J-chain

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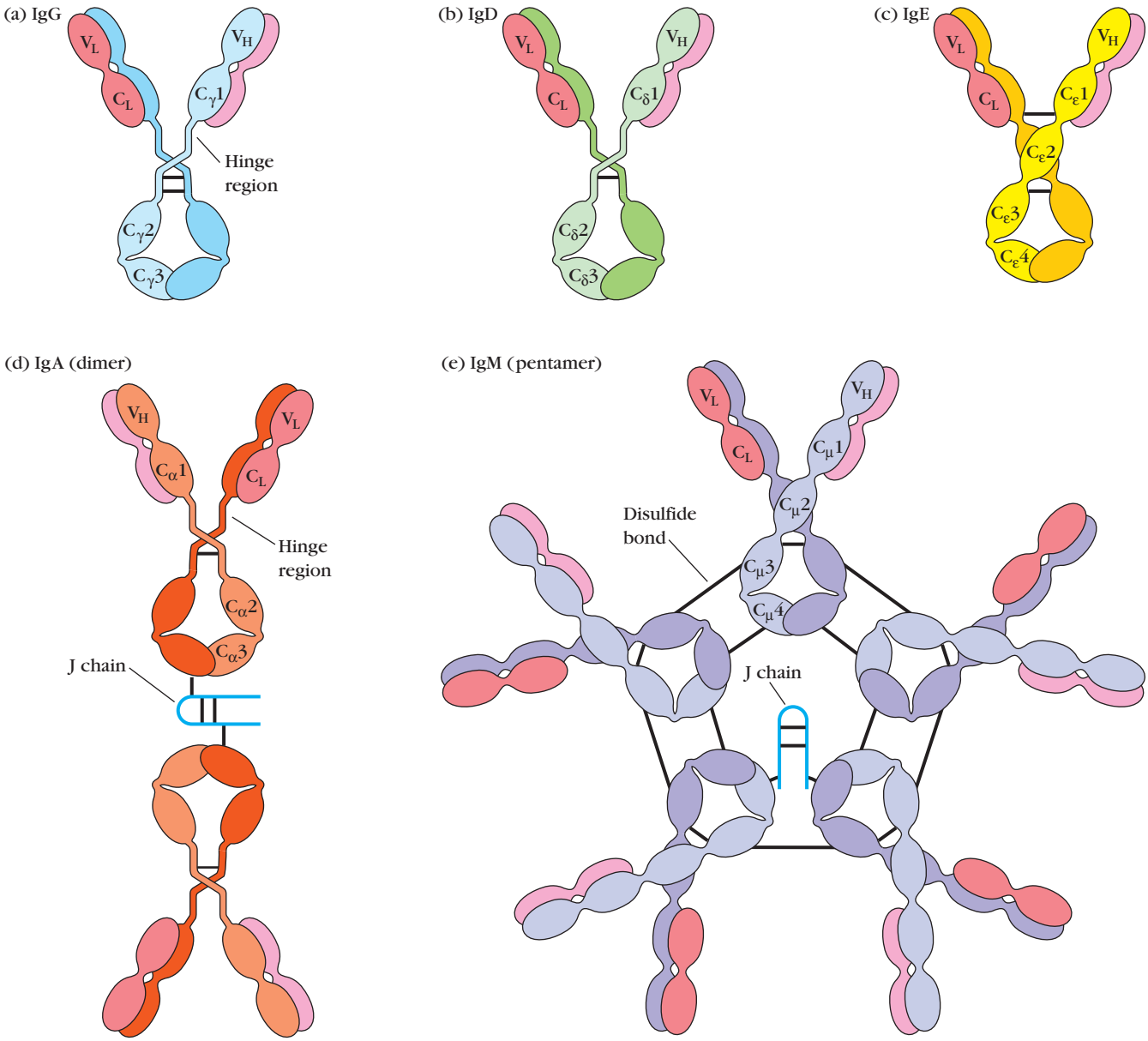

FIGURE 4-13 General structures of the five major classes of secreted antibody. Light chains are shown in shades of pink, disulfide bonds are indicated by thick black lines. Note that the IgG, IgA, and IgD heavy chains (blue, orange, and green, respectively) contain four domains and a hinge region, whereas the IgM and IgE heavy chains (purple and yellow, respectively) contain five domains but no hinge region. The polymeric forms of IgM and IgA contain a polypeptide, called the J chain, that is linked by two disulfide bonds to the Fc region in two different monomers. Serum IgM is always a pentamer; most serum IgA exists as a monomer, although dimers, trimers, and even tetramers are sometimes present. Not shown in these figures are intrachain disulfide bonds and disulfide bonds linking light and heavy chains (see Figure 4-2).

polypeptide (see Figure 4-13d). The IgA of external secretions, called **secretory IgA,** consists of a dimer or tetramer, a J-chain polypeptide, and a polypeptide chain called **secretory component** (Figure 4-15a, page 93). As is explained below, secretory component is derived from the receptor that is responsible for transporting polymeric IgA across cell membranes. The J-chain polypeptide in IgA is identical to that found in pentameric IgM and serves a similar function in facilitating the polymerization of both serum IgA and secretory IgA. The secretory component is a 70,000-MW polypeptide produced by epithelial cells of mucous membranes. It consists of five immunoglobulin-like domains that bind to the Fc region domains of the IgA dimer. This interaction is stabilized by a disulfide bond between the fifth domain of the secretory component and one of the chains of the dimeric IgA.

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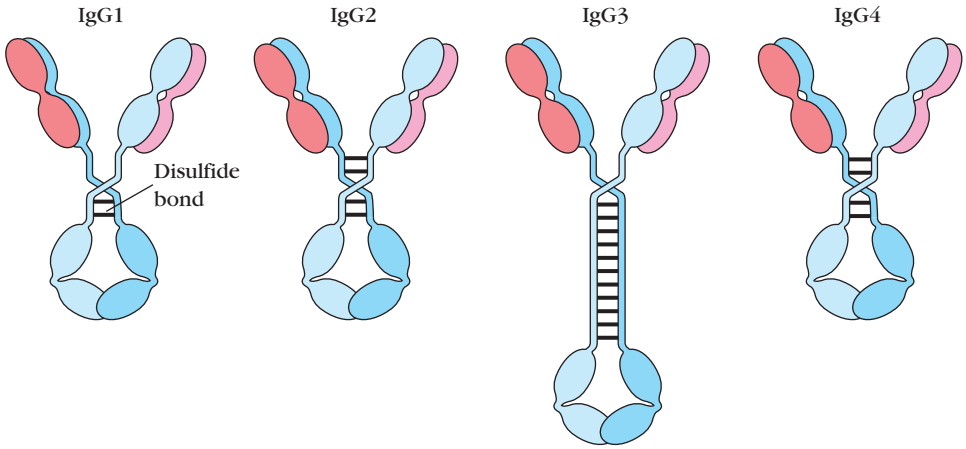

FIGURE 4-14 General structure of the four subclasses of human IgG, which differ in the number and arrangement of the interchain disulfide bonds (thick black lines) linking the heavy chains. A notable feature of human IgG3 is its 11 interchain disulfide bonds.

The daily production of secretory IgA is greater than that of any other immunoglobulin class. IgA-secreting plasma cells are concentrated along mucous membrane surfaces. Along the jejunum of the small intestine, for example, there are more than 2.5×10^{10} IgA-secreting plasma cells—a number that surpasses the total plasma cell population of the bone marrow, lymph, and spleen combined! Every day, a human secretes from 5 g to 15 g of secretory IgA into mucous secretions.

The plasma cells that produce IgA preferentially migrate (home) to subepithelial tissue, where the secreted IgA binds tightly to a receptor for polymeric immunoglobulin molecules (Figure 4-15b). This **poly-Ig receptor** is expressed on the basolateral surface of most mucosal epithelia (e.g., the lining of the digestive, respiratory, and genital tracts) and on glandular epithelia in the mammary, salivary, and lacrimal glands. After polymeric IgA binds to the poly-Ig receptor, the receptor-IgA complex is transported across the epithelial barrier to the lumen. Transport of the receptor-IgA complex involves receptor-mediated endocytosis into coated pits and directed transport of the vesicle across the epithelial cell to the luminal membrane, where the vesicle fuses with the plasma membrane. The poly-Ig receptor is then cleaved enzymatically from the membrane and becomes the secretory component, which is bound to and released together with polymeric IgA into the mucous secretions. The secretory component masks sites susceptible to protease cleavage in the hinge region of secretory IgA, allowing the polymeric molecule to exist longer in the protease-rich mucosal environment than would be possible otherwise. Pentameric IgM is also transported into mucous secretions by this mechanism, although it accounts for a much lower percentage of antibody in the mucous secretions than does IgA. The poly-Ig receptor interacts with the J chain of both polymeric IgA and IgM antibodies.

Secretory IgA serves an important effector function at mucous membrane surfaces, which are the main entry sites for most pathogenic organisms. Because it is polymeric, secretory IgA can cross-link large antigens with multiple epitopes. Binding of secretory IgA to bacterial and viral surface antigens prevents attachment of the pathogens to the mucosal cells, thus inhibiting viral infection and bacterial colonization. Complexes of secretory IgA and antigen are easily entrapped in mucus and then eliminated by the ciliated epithelial cells of the respiratory tract or by peristalsis of the gut. Secretory IgA has been shown to provide an important line of defense against bacteria such as *Salmonella, Vibrio cholerae,* and *Neisseria gonorrhoeae* and viruses such as polio, influenza, and reovirus.

Breast milk contains secretory IgA and many other molecules that help protect the newborn against infection during the first month of life (Table 4-3). Because the immune system of infants is not fully functional, breast-feeding plays an important role in maintaining the health of newborns.

Immunoglobulin E (IgE)

The potent biological activity of IgE allowed it to be identified in serum despite its extremely low average serum concentration (0.3 μg/ml). IgE antibodies mediate the immediate hypersensitivity reactions that are responsible for the symptoms of hay fever, asthma, hives, and anaphylactic shock. The presence of a serum component responsible for allergic reactions was first demonstrated in 1921 by K. Prausnitz and H. Kustner, who injected serum from an allergic person intra-dermally into a nonallergic individual. When the appropriate antigen was later injected at the same site, a wheal and flare reaction (analogous to hives) developed there. This reaction, called the **P-K reaction** (named for its originators, Prausnitz and Kustner), was the basis for the first biological assay for IgE activity.

Actual identification of IgE was accomplished by K. and T. Ishizaka in 1966. They obtained serum from an allergic in-

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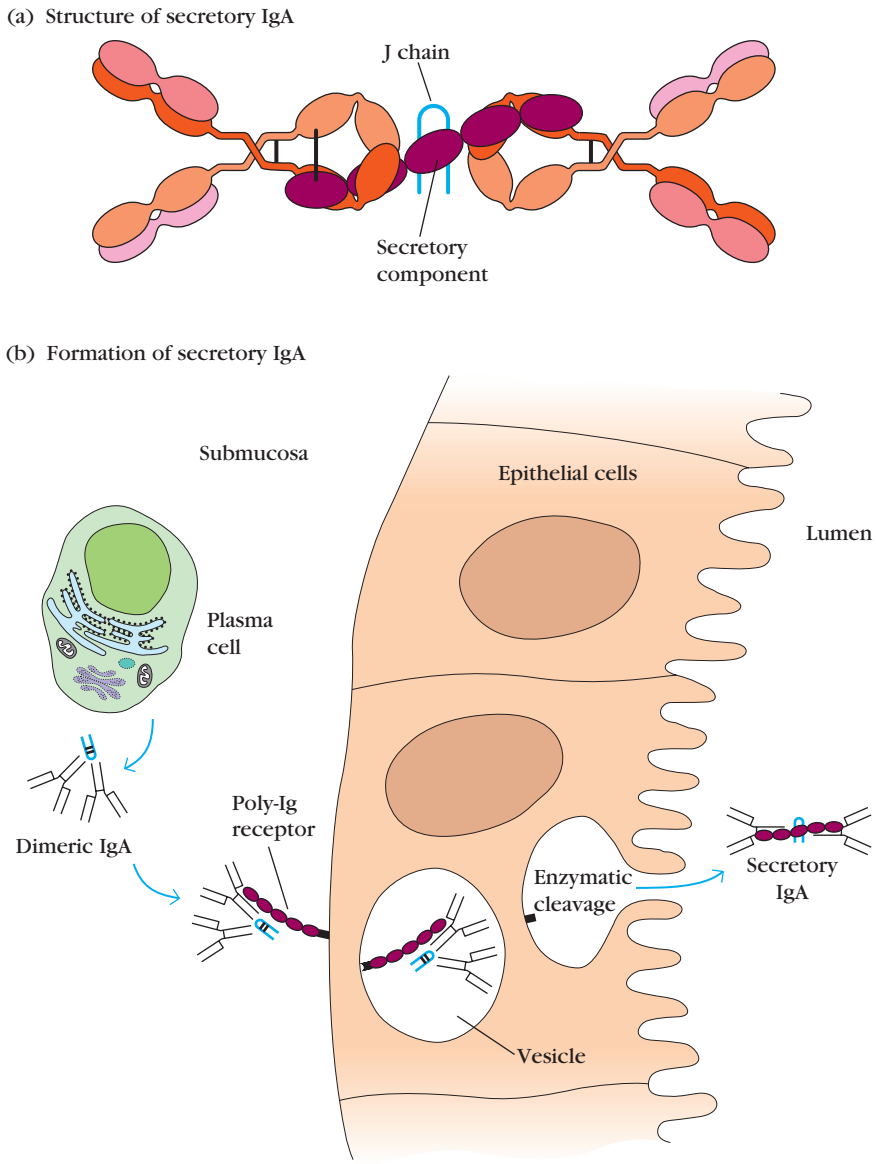

FIGURE 4-15 Structure and formation of secretory IgA. (a) Secretory IgA consists of at least two IgA molecules, which are covalently linked to each other through a J chain and are also covalently linked with the secretory component. The secretory component contains five Ig-like domains and is linked to dimeric IgA by a disulfide bond between its fifth domain and one of the IgA heavy chains. (b) Secretory IgA is formed during transport through mucous membrane epithelial cells. Dimeric IgA binds to a poly-Ig receptor on the basolateral membrane of an epithelial cell and is internalized by receptor-mediated endocytosis. After transport of the receptor-IgA complex to the luminal surface, the poly-Ig receptor is enzymatically cleaved, releasing the secretory component bound to the dimeric IgA.

dividual and immunized rabbits with it to prepare anti-isotype antiserum. The rabbit antiserum was then allowed to react with each class of human antibody known at that time (i.e., IgG, IgA, IgM, and IgD). In this way, each of the known anti-isotype antibodies was precipitated and removed from the rabbit anti-serum. What remained was an anti-isotype antibody specific for an unidentified class of antibody. This antibody turned out to completely block the P-K reaction. The new antibody was called IgE (in reference to the E antigen of ragweed pollen, which is a potent inducer of this class of antibody).

IgE binds to Fc receptors on the membranes of blood basophils and tissue mast cells. Cross-linkage of receptor-bound IgE molecules by antigen (allergen) induces basophils and mast cells to translocate their granules to the plasma membrane and release their contents to the extracellular environment, a process known as degranulation. As a result, a variety of pharmacologically active mediators are released and give rise to allergic manifestations (Figure 4-16). Localized mast-cell degranulation induced by IgE also may release mediators that facilitate a buildup of various cells necessary for antiparasitic defense (see Chapter 15).

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TABLE 4-3 Immune benefits of breast milk

Antibodies of secretory IgA class	Bind to microbes in baby's digestive tract and thereby prevent their attachment to the walls of the gut and their subsequent passage into the body's tissues.
B_{12} binding protein	Reduces amount of vitamin B_{12}, which bacteria need in order to grow.
Bifidus factor	Promotes growth of *Lactobacillus bifidus,* a harmless bacterium, in baby's gut. Growth of such nonpathogenic bacteria helps to crowd out dangerous varieties.
Fatty acids	Disrupt membranes surrounding certain viruses and destroy them.
Fibronectin	Increases antimicrobial activity of macrophages; helps to repair tissues that have been damaged by immune reactions in baby's gut.
Hormones and growth factors	Stimulate baby's digestive tract to mature more quickly. Once the initially "leaky" membranes lining the gut mature, infants become less vulnerable to microorganisms.
Interferon (IFN-γ)	Enhances antimicrobial activity of immune cells.
Lactoferrin	Binds to iron, a mineral many bacteria need to survive. By reducing the available amount of iron, lactoferrin thwarts growth of pathogenic bacteria.
Lysozyme	Kills bacteria by disrupting their cell walls.
Mucins	Adhere to bacteria and viruses, thus keeping such microorganisms from attaching to mucosal surfaces.
Oligosaccharides	Bind to microorganisms and bar them from attaching to mucosal surfaces.

SOURCE: Adapted from J. Newman, 1995, How breast milk protects newborns, *Sci. Am.* **273(6):**76.

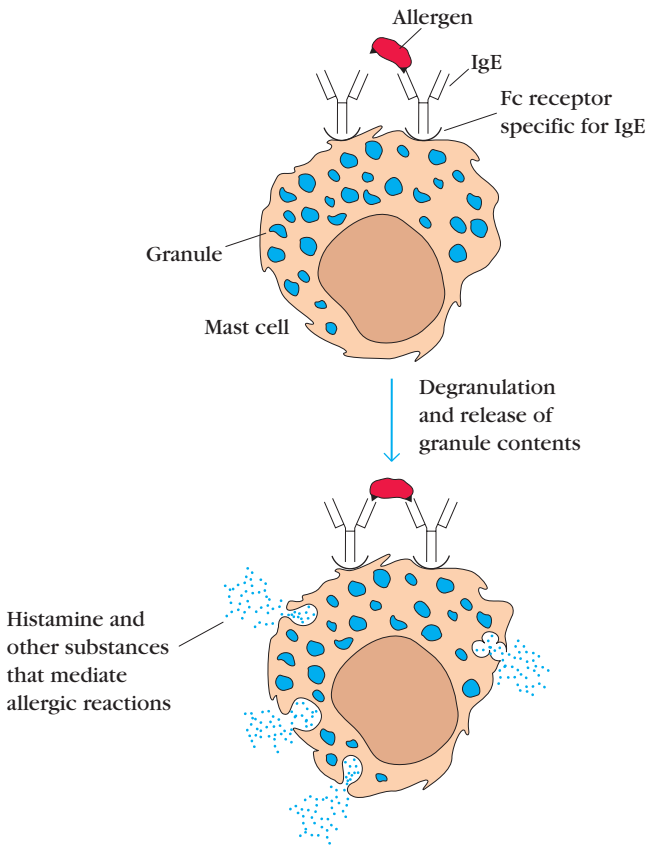

FIGURE 4-16 Allergen cross-linkage of receptor-bound IgE on mast cells induces degranulation, causing release of substances (blue dots) that mediate allergic manifestations.

Immunoglobulin D (IgD)

IgD was first discovered when a patient developed a multiple myeloma whose myeloma protein failed to react with anti-isotype antisera against the then-known isotypes: IgA, IgM, and IgG. When rabbits were immunized with this myeloma protein, the resulting antisera were used to identify the same class of antibody at low levels in normal human serum. The new class, called IgD, has a serum concentration of 30 μg/ml and constitutes about 0.2% of the total immunoglobulin in serum. IgD, together with IgM, is the major membrane-bound immunoglobulin expressed by mature B cells, and its role in the physiology of B cells is under investigation. No biological effector function has been identified for IgD.

Antigenic Determinants on Immunoglobulins

Since antibodies are glycoproteins, they can themselves function as potent immunogens to induce an antibody response. Such anti-Ig antibodies are powerful tools for the study of B-cell development and humoral immune responses. The antigenic determinants, or epitopes, on immunoglobulin molecules fall into three major categories: isotypic, allotypic, and idiotypic determinants, which are located in characteristic portions of the molecule (Figure 4-17).

Isotype

Isotypic determinants are constant-region determinants that collectively define each heavy-chain class and subclass and

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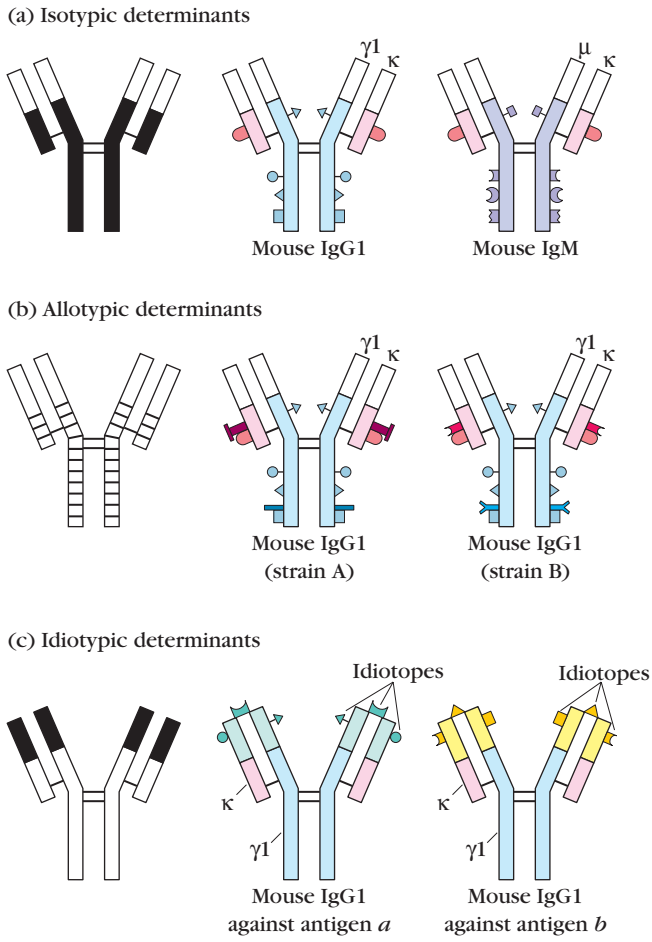

FIGURE 4-17 Antigenic determinants of immunoglobulins. For each type of determinant, the general location of determinants within the antibody molecule is shown (*left*) and two examples are illustrated (*center* and *right*). (a) Isotypic determinants are constant-region determinants that distinguish each Ig class and subclass within a species. (b) Allotypic determinants are subtle amino acid differences encoded by different alleles of isotype genes. Allotypic differences can be detected by comparing the same antibody class among different inbred strains. (c) Idiotypic determinants are generated by the conformation of the amino acid sequences of the heavy- and light-chain variable regions specific for each antigen. Each individual determinant is called an idiotope, and the sum of the individual idiotopes is the idiotype.

each light-chain type and subtype within a species (see Figure 4-17a). Each isotype is encoded by a separate constant-region gene, and all members of a species carry the same constant-region genes (which may include multiple alleles). Within a species, each normal individual will express all isotypes in the serum. Different species inherit different constant-region genes and therefore express different isotypes. Therefore, when an antibody from one species is injected into another species, the isotypic determinants will be recognized as foreign, inducing an antibody response to the isotypic determinants on the foreign antibody. Anti-isotype antibody is routinely used for research purposes to determine the class or subclass of serum antibody produced during an immune response or to characterize the class of membrane-bound antibody present on B cells.

Allotype

Although all members of a species inherit the same set of isotype genes, multiple alleles exist for some of the genes (see Figure 4-17b). These alleles encode subtle amino acid differences, called allotypic determinants, that occur in some, but not all, members of a species. The sum of the individual allotypic determinants displayed by an antibody determines its **allotype.** In humans, allotypes have been characterized for all four IgG subclasses, for one IgA subclass, and for the κ light chain. The γ-chain allotypes are referred to as Gm markers. At least 25 different Gm allotypes have been identified; they are designated by the class and subclass followed by the allele number, for example, G1m(1), G2m(23), G3m(11), G4m(4a). Of the two IgA subclasses, only the IgA2 subclass has allotypes, as A2m(1) and A2m(2). The κ light chain has three allotypes, designated κm(1), κm(2), and κm(3). Each of these allotypic determinants represents differences in one to four amino acids that are encoded by different alleles.

Antibody to allotypic determinants can be produced by injecting antibodies from one member of a species into another member of the same species who carries different allotypic determinants. Antibody to allotypic determinants sometimes is produced by a mother during pregnancy in response to paternal allotypic determinants on the fetal immunoglobulins. Antibodies to allotypic determinants can also arise from a blood transfusion.

Idiotype

The unique amino acid sequence of the V_H and V_L domains of a given antibody can function not only as an antigen-binding site but also as a set of antigenic determinants. The idiotypic determinants arise from the sequence of the heavy- and light-chain variable regions. Each individual antigenic determinant of the variable region is referred to as an **idiotope** (see Figure 4-17c). In some cases an idiotope may be the actual antigen-binding site, and in some cases an idiotope may comprise variable-region sequences outside of the antigen-binding site. Each antibody will present multiple idiotopes; the sum of the individual idiotopes is called the **idiotype** of the antibody.

Because the antibodies produced by individual B cells derived from the same clone have identical variable-region sequences, they all have the same idiotype. Anti-idiotype antibody is produced by injecting antibodies that have minimal variation in their isotypes and allotypes, so that the idiotypic difference can be recognized. Often a homogeneous antibody such as myeloma protein or monoclonal antibody is used. Injection of such an antibody into a recipient who is

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genetically identical to the donor will result in the formation of anti-idiotype antibody to the idiotypic determinants.

The B-Cell Receptor

Immunologists have long been puzzled about how mIg mediates an activating signal after contact with an antigen. The dilemma is that all isotypes of mIg have very short cytoplasmic tails: the mIgM and mIgD cytoplasmic tails contain only 3 amino acids; the mIgA tail, 14 amino acids; and the mIgG and mIgE tails, 28 amino acids. In each case, the cytoplasmic tail is too short to be able to associate with intracellular signaling molecules (e.g., tyrosine kinases and G proteins).

The answer to this puzzle is that mIg does not constitute the entire antigen-binding receptor on B cells. Rather, the **B-cell receptor (BCR)** is a transmembrane protein complex composed of mIg and disulfide-linked heterodimers called Ig-α/Ig-β. Molecules of this heterodimer associate with an mIg molecule to form a BCR (Figure 4-18). The Ig-α chain has a long cytoplasmic tail containing 61 amino acids; the tail of the Ig-β chain contains 48 amino acids. The tails in both Ig-α and Ig-β are long enough to interact with intracellular signaling molecules. Discovery of the Ig-α/Ig-β heterodimer by Michael Reth and his colleagues in the early 1990s has substantially furthered understanding of B-cell activation, which is discussed in detail in Chapter 11.

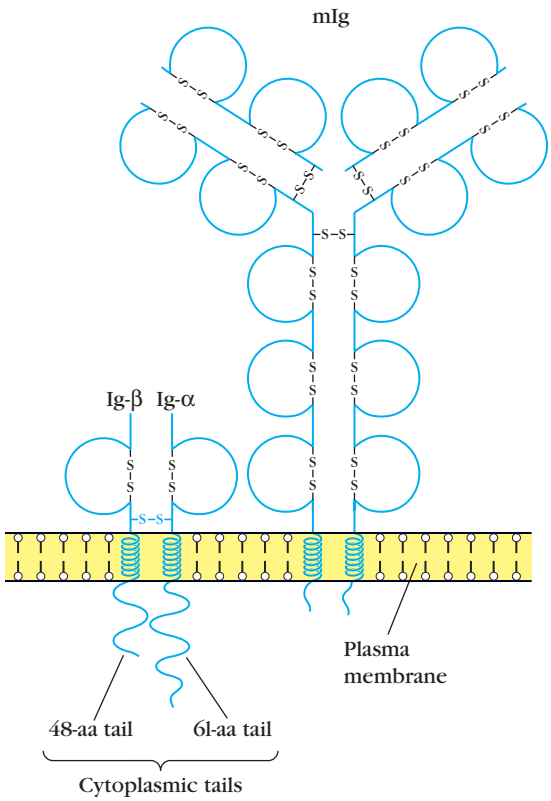

FIGURE 4-18 General structure of the B-cell receptor (BCR). This antigen-binding receptor is composed of membrane-bound immunoglobulin (mIg) and disulfide-linked heterodimers called Ig-α/Ig-β. Each heterodimer contains the immunoglobulin-fold structure and cytoplasmic tails much longer than those of mIg. As depicted, an mIg molecule is associated with one Ig-α/Ig-β heterodimer. *[Adapted from A. D. Keegan and W. E. Paul, 1992,* Immunol. Today ***13:****63, and M. Reth, 1992,* Annu. Rev. Immunol. ***10:****97.]*

Fc Receptors Bond to Fc Regions of Antibodies

Many cells feature membrane glycoproteins called **Fc receptors (FcR)** that have an affinity for the Fc portion of the antibody molecule. These receptors are essential for many of the biological functions of antibodies. Fc receptors are responsible for the movement of antibodies across cell membranes and the transfer of IgG from mother to fetus across the placenta. These receptors also allow passive acquisition of antibody by many cell types, including B and T lymphocytes, neutrophils, mast cells, eosinophils, macrophages, and natural killer cells. Consequently, Fc receptors provide a means by which antibodies—the products of the adaptive immune system—can recruit such key cellular elements of innate immunity as macrophages and natural killer cells. Engagement of antibody-bound antigens by the Fc receptors of macrophages or neutrophils provides an effective signal for the efficient phagocytosis (opsonization) of antigen-antibody complexes. In addition to triggering such effector functions as opsonization or ADCC, crosslinking of Fc receptors by antigen-mediated crosslinking of FcR-bound antibodies can generate immunoregulatory signals that affect cell activation, induce differentiation and, in some cases, downregulate cellular responses.

There are many different Fc receptors (Figure 4-19). The poly Ig receptor is essential for the transport of polymeric immunoglobulins (polymeric IgA and to some extent, pentameric IgM) across epithelial surfaces. In humans, the **neonatal Fc receptor (FcR_N)** transfers IgGs from mother to fetus during gestation and also plays a role in the regulation of IgG serum levels. Fc receptors have been discovered for all of the Ig classes. Thus there is an FcαR receptor that binds IgA, an FcεR that binds IgE (see Figure 4-16 also), an FcδR that binds IgD, IgM is bound by an FcμR, and several varieties of FcγR receptors capable of binding IgG and its subclasses are found in humans. In many cases, the crosslinking of these receptors by binding of antigen-antibody complexes results in the initiation of signal-transduction cascades that result in such behaviors as phagocytosis or ADCC. The Fc receptor is often part of a signal-transducing complex that involves the participation of other accessory polypeptide chains. As shown in Figure 4-19, this may involve a pair of γ chains or, in the case of the IgE receptor, a more complex assemblage of two γ chains and a β chain. The association of an extracellular receptor with an intracellular signal-transducing unit was seen in the B cell receptor (Figure 4-18) and is a central feature of the T-cell-receptor complex (Chapter 9).

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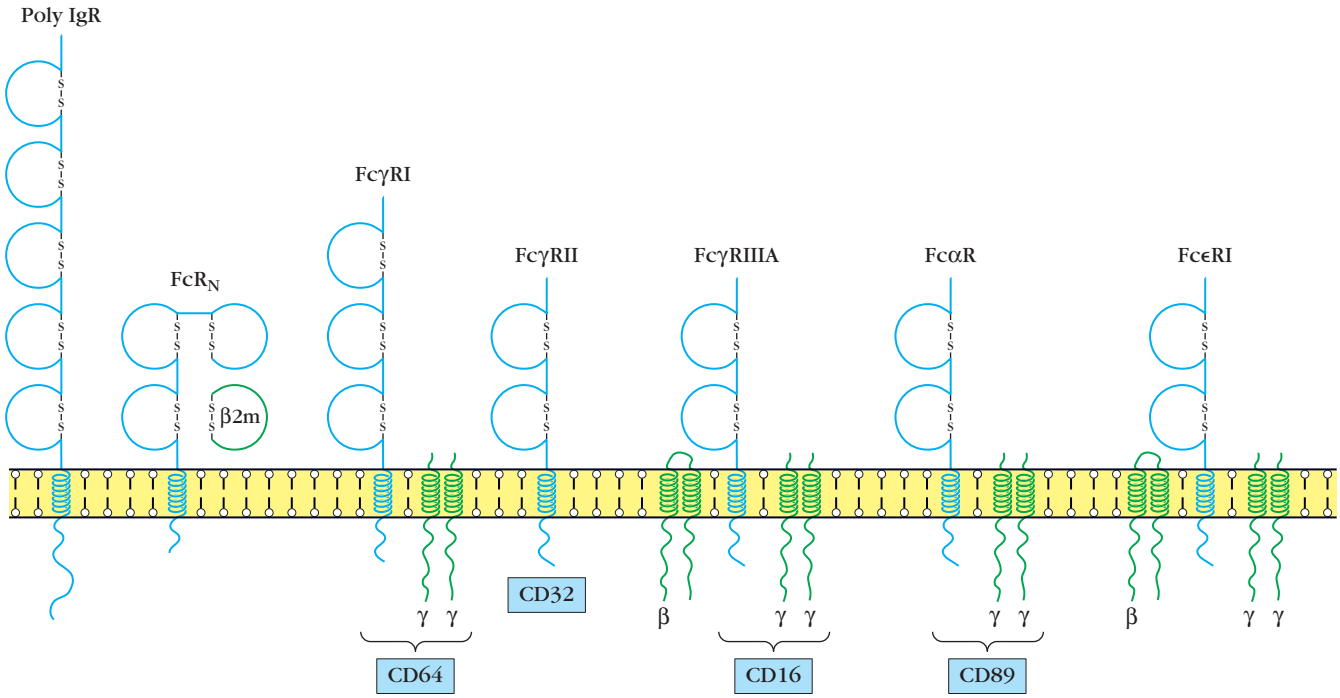

FIGURE 4-19 The structure of a number of human Fc-receptors. The Fc-binding polypeptides are shown in blue and, where present, accessory signal-transducing polypeptides are shown in green. The loops in these structures represent portions of the molecule with the characteristic immunoglobulin-fold structure. These molecules appear on the plasma membrane as cell-surface antigens and, as indicated in the figure, many have been assigned CD designations (for clusters of differentiation; see Appendix). *[Adapted from M. Daeron, 1999, in* The Antibodies, *vol. 5, p. 53. Edited by M. Zanetti and J. D. Capra.]*

The Immunoglobulin Superfamily

The structures of the various immunoglobulin heavy and light chains described earlier share several features, suggesting that they have a common evolutionary ancestry. In particular, all heavy- and light-chain classes have the immunoglobulin-fold domain structure (see Figure 4-7). The presence of this characteristic structure in all immunoglobulin heavy and light chains suggests that the genes encoding them arose from a common primordial gene encoding a polypeptide of about 110 amino acids. Gene duplication and later divergence could then have generated the various heavy- and light-chain genes.

Large numbers of membrane proteins have been shown to possess one or more regions homologous to an immunoglobulin domain. Each of these membrane proteins is classified as a member of the **immunoglobulin superfamily.** The term *superfamily* is used to denote proteins whose corresponding genes derived from a common primordial gene encoding the basic domain structure. These genes have evolved independently and do not share genetic linkage or function. The following proteins, in addition to the immunoglobulins themselves, are representative members of the immunoglobulin superfamily (Figure 4-20):

- Ig-α/Ig-β heterodimer, part of the B-cell receptor
- Poly-Ig receptor, which contributes the secretory component to secretory IgA and IgM
- T-cell receptor
- T-cell accessory proteins, including CD2, CD4, CD8, CD28, and the γ, δ, and ε chains of CD3
- Class I and class II MHC molecules
- β_2-microglobulin, an invariant protein associated with class I MHC molecules
- Various cell-adhesion molecules, including VCAM-1, ICAM-1, ICAM-2, and LFA-3
- Platelet-derived growth factor

Numerous other proteins, some of them discussed in other chapters, also belong to the immunoglobulin superfamily.

X-ray crystallographic analysis has not been accomplished for all members of the immunoglobulin superfamily. Nevertheless, the primary amino acid sequence of these proteins suggests that they all contain the typical immunoglobulin-fold domain. Specifically, all members of the immunoglobulin superfamily contain at least one or more stretches of about 110 amino acids, capable of arrangement

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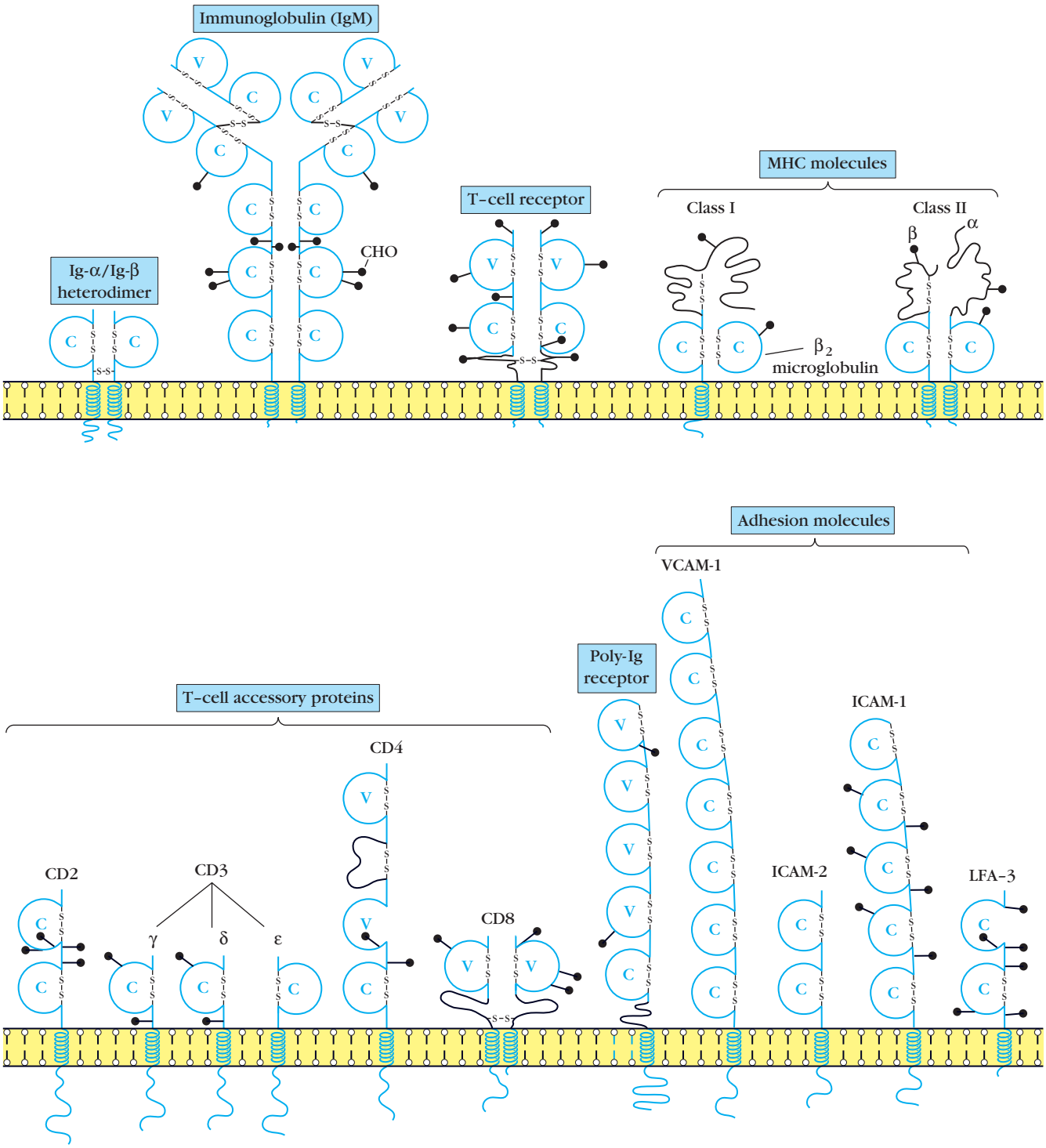

FIGURE 4-20 Some members of the immunoglobulin superfamily, a group of structurally related, usually membrane-bound glycoproteins. In all cases shown here except for β_2-microglobulin, the carboxyl-terminal end of the molecule is anchored in the membrane.

into pleated sheets of antiparallel β strands, usually with an invariant intrachain disulfide bond that closes a loop spanning 50–70 residues.

Most members of the immunoglobulin superfamily cannot bind antigen. Thus, the characteristic Ig-fold structure found in so many membrane proteins must have some function other than antigen binding. One possibility is that the immunoglobulin fold may facilitate interactions between membrane proteins. As described earlier, interactions can occur between the faces of β pleated sheets both of homolo-

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gous immunoglobulin domains (e.g., C_H2/C_H2 interaction) and of nonhomologous domains (e.g., V_H/V_L and C_H1/C_L interactions).

Monoclonal Antibodies

As noted in Chapter 3, most antigens offer multiple epitopes and therefore induce proliferation and differentiation of a variety of B-cell clones, each derived from a B cell that recognizes a particular epitope. The resulting serum antibodies are heterogeneous, comprising a mixture of antibodies, each specific for one epitope (Figure 4-21). Such a **polyclonal antibody** response facilitates the localization, phagocytosis, and complement-mediated lysis of antigen; it thus has clear advantages for the organism in vivo. Unfortunately, the antibody heterogeneity that increases immune protection in vivo often reduces the efficacy of an antiserum for various in vitro uses. For most research, diagnostic, and therapeutic purposes, **monoclonal antibodies,** derived from a single clone and thus specific for a single epitope, are preferable.

Direct biochemical purification of a monoclonal antibody from a polyclonal antibody preparation is not feasible. In 1975, Georges Köhler and Cesar Milstein devised a method for preparing monoclonal antibody, which quickly became one of immunology's key technologies. By fusing a normal activated, antibody-producing B cell with a myeloma cell (a cancerous plasma cell), they were able to generate a hybrid cell, called a **hybridoma,** that possessed the immortal-growth properties of the myeloma cell and secreted the

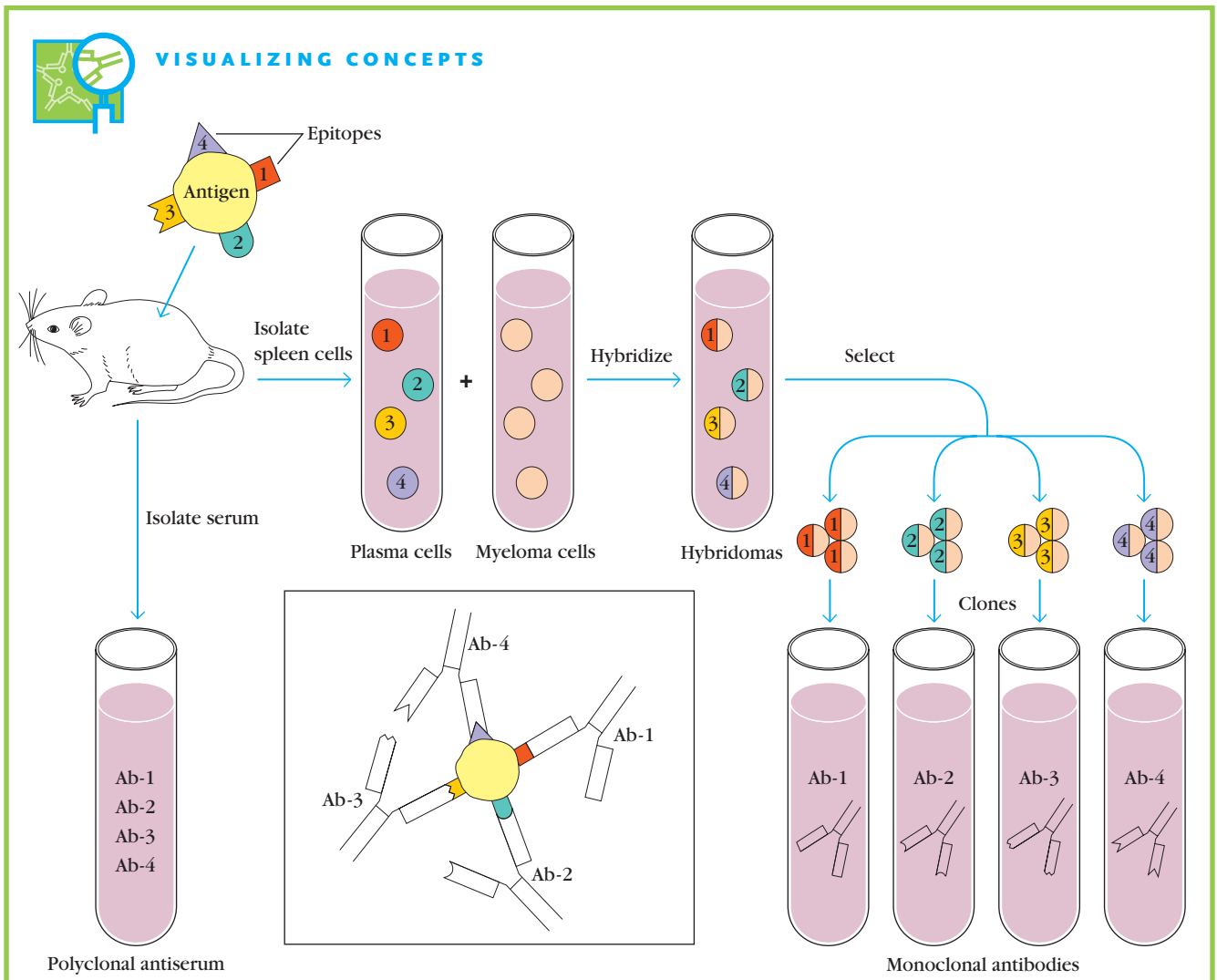

FIGURE 4-21 The conventional polyclonal antiserum produced in response to a complex antigen contains a mixture of monoclonal antibodies, each specific for one of the four epitopes shown on the antigen (inset). In contrast, a monoclonal antibody, which is derived from a single plasma cell, is specific for one epitope on a complex antigen. The outline of the basic method for obtaining a monoclonal antibody is illustrated here.

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antibody produced by the B cell (see Figure 4-21). The resulting clones of hybridoma cells, which secrete large quantities of monoclonal antibody, can be cultured indefinitely. The development of techniques for producing monoclonal antibodies, the details of which are discussed in Chapter 23, gave immunologists a powerful and versatile research tool. The significance of the work by Köhler and Milstein was acknowledged when each was awarded a Nobel Prize.

Monoclonal Antibodies Have Important Clinical Uses

Monoclonal antibodies are proving to be very useful as diagnostic, imaging, and therapeutic reagents in clinical medicine. Initially, monoclonal antibodies were used primarily as in vitro diagnostic reagents. Among the many monoclonal antibody diagnostic reagents now available are products for detecting pregnancy, diagnosing numerous pathogenic microorganisms, measuring the blood levels of various drugs, matching histocompatibility antigens, and detecting antigens shed by certain tumors.

Radiolabeled monoclonal antibodies can also be used in vivo for detecting or locating tumor antigens, permitting earlier diagnosis of some primary or metastatic tumors in patients. For example, monoclonal antibody to breast-cancer cells is labeled with iodine-131 and introduced into the blood to detect the spread of a tumor to regional lymph nodes. This monoclonal imaging technique can reveal breast-cancer metastases that would be undetected by other, less sensitive scanning techniques.

Immunotoxins composed of tumor-specific monoclonal antibodies coupled to lethal toxins are potentially valuable therapeutic reagents. The toxins used in preparing immunotoxins include ricin, *Shigella* toxin, and diphtheria toxin, all of which inhibit protein synthesis. These toxins are so potent that a single molecule has been shown to kill a cell. Each of these toxins consists of two types of functionally distinct polypeptide components, an inhibitory (toxin) chain and one or more binding chains, which interact with receptors on cell surfaces; without the binding polypeptide(s) the toxin cannot get into cells and therefore is harmless. An immunotoxin is prepared by replacing the binding polypeptide(s) with a monoclonal antibody that is specific for a particular tumor cell (Figure 4-22a). In theory, the attached monoclonal antibody will deliver the toxin chain specifically to tumor cells, where it will cause death by inhibiting protein synthesis (Figure 4-22b). The initial clinical responses to such immunotoxins in patients with leukemia, lymphoma, and some other types of cancer have shown promise, and research to develop and demonstrate their safety and effectiveness is underway.

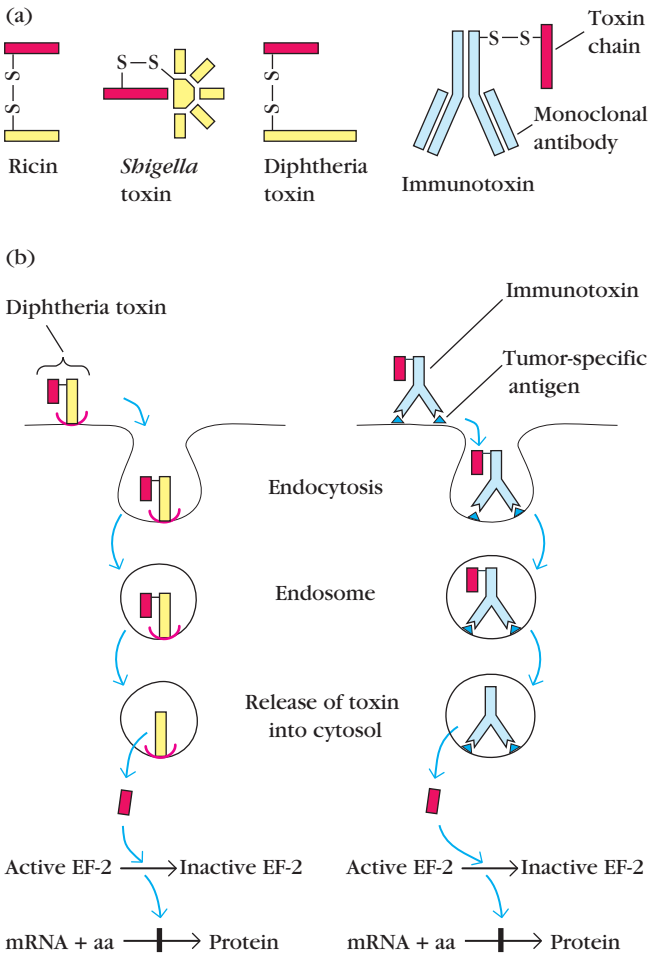

FIGURE 4-22 (a) Toxins used to prepare immunotoxins include ricin, *Shigella* toxin, and diphtheria toxin. Each toxin contains an inhibitory toxin chain (red) and a binding component (yellow). To make an immunotoxin, the binding component of the toxin is replaced with a monoclonal antibody (blue). (b) Diphtheria toxin binds to a cell-membrane receptor (*left*) and a diphtheria-immunotoxin binds to a tumor-associated antigen (*right*). In either case, the toxin is internalized in an endosome. The toxin chain is then released into the cytoplasm, where it inhibits protein synthesis by catalyzing the inactivation of elongation factor 2 (EF-2).

Abzymes Are Monoclonal Antibodies That Catalyze Reactions

The binding of an antibody to its antigen is similar in many ways to the binding of an enzyme to its substrate. In both cases the binding involves weak, noncovalent interactions and exhibits high specificity and often high affinity. What distinguishes an antibody-antigen interaction from an enzyme-substrate interaction is that the antibody does not alter the antigen, whereas the enzyme catalyzes a chemical change in its substrate. However, like enzymes, antibodies of appropriate specificity can stabilize the transition state of a bound substrate, thus reducing the activation energy for chemical modification of the substrate.

The similarities between antigen-antibody interactions and enzyme-substrate interactions raised the question of whether some antibodies could behave like enzymes and catalyze chemical reactions. To investigate this possibility, a

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hapten-carrier complex was synthesized in which the hapten structurally resembled the transition state of an ester undergoing hydrolysis. Spleen cells from mice immunized with this transition state analogue were fused with myeloma cells to generate monoclonal antihapten monoclonal antibodies. When these monoclonal antibodies were incubated with an ester substrate, some of them accelerated hydrolysis by about 1000-fold; that is, they acted like the enzyme that normally catalyzes the substrate's hydrolysis. The catalytic activity of these antibodies was highly specific; that is, they hydrolyzed only esters whose transition-state structure closely resembled the transition state analogue used as a hapten in the immunizing conjugate. These catalytic antibodies have been called **abzymes** in reference to their dual role as antibody and enzyme.

A central goal of catalytic antibody research is the derivation of a battery of abzymes that cut peptide bonds at specific amino acid residues, much as restriction enzymes cut DNA at specific sites. Such abzymes would be invaluable tools in the structural and functional analysis of proteins. Additionally, it may be possible to generate abzymes with the ability to dissolve blood clots or to cleave viral glycoproteins at specific sites, thus blocking viral infectivity. Unfortunately, catalytic antibodies that cleave the peptide bonds of proteins have been exceedingly difficult to derive. Much of the research currently being pursued in this field is devoted to the solution of this important but difficult problem.

SUMMARY

- An antibody molecule consists of two identical light chains and two identical heavy chains, which are linked by disulfide bonds. Each heavy chain has an amino-terminal variable region followed by a constant region.
- In any given antibody molecule, the constant region contains one of five basic heavy-chain sequences (μ, γ, δ, α, or ϵ) called isotypes and one of two basic light-chain sequences (κ or λ) called types.
- The heavy-chain isotype determines the class of an antibody (μ, IgM; γ, IgG; δ, IgD; α, IgA; and ϵ, IgE).
- The five antibody classes have different effector functions, average serum concentrations, and half-lives.
- Each of the domains in the immunoglobulin molecule has a characteristic tertiary structure called the immunoglobulin fold. The presence of an immunoglobulin fold domain also identifies many other nonantibody proteins as members of the immunoglobulin superfamily.
- Within the amino-terminal variable domain of each heavy and light chain are three complementarity-determining regions (CDRs). These polypeptide regions contribute the antigen-binding site of an antibody, determining its specificity.
- Immunoglobulins are expressed in two forms: secreted antibody that is produced by plasma cells, and membrane-bound antibody that associates with Ig-α/Ig-β heterodimers to form the B-cell antigen receptor present on the surface of B cells.
- The three major effector functions that enable antibodies to remove antigens and kill pathogens are: opsonization, which promotes antigen phagocytosis by macrophages and neutrophils; complement activation, which activates a pathway that leads to the generation of a collection of proteins that can perforate cell membranes; and antibody-dependent cell-mediated cytotoxicity (ADCC), which can kill antibody-bound target cells.
- Unlike polyclonal antibodies that arise from many B cell clones and have a heterogeneous collection of binding sites, a monoclonal antibody is derived from a single B cell clone and is a homogeneous collection of binding sites.

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USEFUL WEB SITES

http://immuno.bme.nwu.edu/

The Kabat Database of Sequences of Proteins of Immunological Interest: This site has the amino acid and DNA sequences of many antibodies and other proteins that play important roles in immunology.

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http://www.biochem.ucl.ac.uk/~martin/abs

Antibodies—Structure and Sequence: This Web site summarizes useful information on antibody structure and sequence. It provides general information on antibodies and crystal structures and links to other antibody-related information.

http://www.ncbi.nlm.nih.gov

National Center for Biotechnology Information (NCBI): A unique and comprehensive resource of computerized databases of bibliographic information, nucleic acid sequences, protein sequences, and sequence analysis tools created and maintained by the National Library of Medicine.

http://www.ncbi.nlm.nih.gov/Structure/

The Molecular Modeling Database (MMDB) contains 3-dimensional structures determined by x-ray crystallography and NMR spectroscopy. The data for MMDB are obtained from the Protein Data Bank (PDB). The National Center for Biotechnology Information (NCBI) has structural data crosslinked to bibliographic information, to databases of protein and nucleic acid sequences, and to the NCBI animal taxonomy database. The NCBI has developed a 3D structure viewer, Cn3D, for easy interactive visualization of molecular structures.

http://www.umass.edu/microbio/chime/explorer/

Protein Explorer is a molecular visualization program created by Eric Martz with the support of the National Science Foundation to make it easier for students, educators, and scientists to use interactive and dynamic molecular visualization techniques. Many will find it easier to use than Chime and Rasmol.

http://imgt.cines.fr

IMGT, the international ImMunoGeneTics database created by Marie-Paule Lefranc, is a well organized, powerful, and comprehensive information system that specializes in immunoglobulins, T-cell receptors and major histocompatibility complex (MHC) molecules of all vertebrate species.

Study Questions

CLINICAL FOCUS QUESTION Two pharmaceutical companies make IVIG. Company A produces their product from pools of 100,000 donors drawn exclusively from the population of the United States. Company B makes their IVIG from pools of 60,000 donors drawn in equal numbers from North America, Europe, Brazil, and Japan.

a. Which product would you expect to have the broadest spectrum of pathogen reactivities? Why?
b. Assume the patients receiving the antibody will (1) never leave the USA, or (2) travel extensively in many parts of the world. Which company's product would you choose for each of these patient groups? Justify your choices.

1. Indicate whether each of the following statements is true or false. If you think a statement is false, explain why.

a. A rabbit immunized with human IgG3 will produce antibody that reacts with all subclasses of IgG in humans.
b. All immunoglobulin molecules on the surface of a given B cell have the same idiotype.
c. All immunoglobulin molecules on the surface of a given B cell have the same isotype.
d. All myeloma protein molecules derived from a single myeloma clone have the same idiotype and allotype.
e. Although IgA is the major antibody species that undergoes transcytosis, polymeric IgM, but not monomeric IgA, can also undergo transcytosis.
f. The hypervariable regions make significant contact with the epitope.
g. IgG functions more effectively than IgM in bacterial agglutination.
h. Although monoclonal antibodies are often preferred for research and diagnostic purposes, both monoclonal and polyclonal antibodies can be highly specific.
i. All isotypes are normally found in each individual of a species.
j. The heavy-chain variable region (V_H) is twice as long as the light-chain variable region (V_L).

2. You are an energetic immunology student who has isolated protein X, which you believe is a new isotype of human immunoglobulin.

a. What structural features would protein X have to have in order to be classified as an immunoglobulin?
b. You prepare rabbit antisera to whole human IgG, human κ chain, and human γ chain. Assuming protein X is, in fact, a new immunoglobulin isotype, to which of these antisera would it bind? Why?
c. Devise an experimental procedure for preparing an antiserum that is specific for protein X.

3. According to the clonal selection theory, all the immunoglobulin molecules on a single B cell have the same antigenic specificity. Explain why the presence of both IgM and IgD on the same B cell does not violate the unispecificity implied by clonal selection.

4. IgG, which contains γ heavy chains, developed much more recently during evolution than IgM, which contains μ heavy chains. Describe two advantages and two disadvantages that IgG has in comparison with IgM.

5. Although the five immunoglobulin isotypes share many common structural features, the differences in their structures affect their biological activities.

a. Draw a schematic diagram of a typical IgG molecule and label each of the following parts: H chains, L chains, interchain disulfide bonds, intrachain disulfide bonds, hinge, Fab, Fc, and all the domains. Indicate which domains are involved in antigen binding.
b. How would you have to modify the diagram of IgG to depict an IgA molecule isolated from saliva?
c. How would you have to modify the diagram of IgG to depict serum IgM?

6. Fill out the accompanying table relating to the properties of IgG molecules and their various parts. Insert a (+) if the molecule or part exhibits the property; a (−) if it does not; and a (+/−) if it does so only weakly.

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Property	Whole IgG	H chain	L chain	Fab	$F(ab')_2$	Fc
Binds antigen						
Bivalent antigen binding						
Binds to Fc receptors						
Fixed complement in presence of antigen						
Has V domains						
Has C domains						

7. Because immunoglobulin molecules possess antigenic determinants, they themselves can function as immunogens, inducing formation of antibody. For each of the following immunization scenarios, indicate whether anti-immunoglobulin antibodies would be formed to isotypic (IS), allotypic (AL), or idiotypic (ID) determinants:

a. Anti-DNP antibodies produced in a BALB/c mouse are injected into a C57BL/6 mouse.
b. Anti-BGG monoclonal antibodies from a BALB/c mouse are injected into another BALB/c mouse.
c. Anti-BGG antibodies produced in a BALB/c mouse are injected into a rabbit.
d. Anti-DNP antibodies produced in a BALB/c mouse are injected into an outbred mouse.
e. Anti-BGG antibodies produced in a BALB/c mouse are injected into the same mouse.

8. Write YES or NO in the accompanying table to indicate whether the rabbit antisera listed at the top react with the mouse antibody components listed at the left.

	γ chain	κ chain	IgG Fab fragment	IgG Fc fragment	J chain
Mouse γ chain					
Mouse κ chain					
Mouse IgM whole					
Mouse IgM Fc fragment					

9. The characteristic structure of immunoglobulin domains, termed the immunoglobulin fold, also occurs in the numerous membrane proteins belonging to the immunoglobulin superfamily.

a. Describe the typical features that define the immunoglobulin-fold domain structure.
b. Consider proteins that belong to the immunoglobulin superfamily. What do all of these proteins have in common? Describe two different Ig superfamily members that bind antigen. Identify four different Ig superfamily members that do not bind antigen.

10. Where are the CDR regions located on an antibody molecule and what are their functions?

11. The variation in amino acid sequence at each position in a polypeptide chain can be expressed by a quantity termed the variability. What are the largest and smallest values of variability possible?

12. You prepare an immunotoxin by conjugating diphtheria toxin with a monoclonal antibody specific for a tumor antigen.

a. If this immunotoxin is injected into an animal, will any normal cells be killed? Explain.
b. If the antibody part of the immunotoxin is degraded so that the toxin is released, will normal cells be killed? Explain.

13. An investigator wanted to make a rabbit antiserum specific for mouse IgG. She injected a rabbit with purified mouse IgG and obtained an antiserum that reacted strongly with mouse IgG. To her dismay, however, the antiserum also reacted with each of the other mouse isotypes. Explain why she got this result. How could she make the rabbit antiserum specific for mouse IgG?

14. You fuse spleen cells having a normal genotype for immunoglobulin heavy chains (H) and light chains (L) with three myeloma-cell preparations differing in their immunoglobulin genotype as follows: (a) H^+, L^+; (b) H^-, L^+; and (c) H^-, L^-. For each hybridoma, predict how many unique antigen-binding sites, composed of one H and one L chain, theoretically could be produced and show the chain structure of the possible antibody molecules. For each possible antibody molecule indicate whether the chains would originate from the spleen (S) or from the myeloma (M) fusion partner (e.g., H_SL_S/H_mL_m).

15. For each immunoglobulin isotype (a–e) select the description(s) listed below (1–12) that describe that isotype. Each description may be used once, more than once, or not at all; more than one description may apply to some isotypes.

Isotypes

a. _____ IgA
b. _____ IgD
c. _____ IgE
d. _____ IgG
e. _____ IgM

Descriptions

(1) Secreted form is a pentamer of the basic H_2L_2 unit
(2) Binds to Fc receptors on mast cells

(3) Multimeric forms have a J chain
(4) Present on the surface of mature, unprimed B cells
(5) The most abundant isotype in serum
(6) Major antibody in secretions such as saliva, tears, and breast milk
(7) Present on the surface of immature B cells
(8) The first serum antibody made in a primary immune response
(9) Plays an important role in immediate hypersensitivity
(10) Plays primary role in protecting against pathogens that invade through the gut or respiratory mucosa
(11) Multimeric forms may contain a secretory component
(12) Least abundant isotype in serum

16. Describe four distinct roles played by Fc receptors. In what ways is signal transduction from Fc receptors similar to signal transduction from the B-cell receptor?

17. What is IVIG and what are some of the mechanisms by which it might protect the body against infection? Suppose one had the option of collecting blood for the manufacture of IVIG from the following groups of healthy individuals: 35-year-old men who had lived all of their lives in isolated villages in the mountains of Switzerland, or 45–55-year-old men who had been international airline pilots for 20 years. Which group would provide the better pool of blood? Justify your answer.

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