

ANTIBODY STRUCTURE AND FUNCTION

CHAPTER OUTLINE

1. The structure of an antibody is related to its function.

- Studies by Tiselius and Kabat and later by Edelman, Porter, and Nisonoff determined the basic structural components of antibodies.
 - Initially, antibodies were identified only by their electrical charge.
 - Fragmentation studies showed that antibodies are made up of two identical light chains and two identical heavy chains.
- Further studies explained the fine structure of antibodies—the variable and constant regions on the chains.
- The antibody's antigenic determinants—called isotypes, allotypes, and idiotypes—determine the variability in antibody structure.
 - Isotypes are variants present in all members of a species.
 - Allotypes are variants caused by intraspecies genetic differences.
 - Idiotypes are variants caused by structural heterogeneity in the antibody V regions.

2. There are five classes of antibodies based on the structure of their heavy-chain C domains.

- All antibody classes have either λ or κ light chains.
- IgG is the major antibody in the blood, but it is able to enter tissue spaces and coat antigens, speeding antigen uptake.
- IgA concentrates in body fluids to guard the entrances of the body.
- IgM is the largest antibody; it tends to remain in the blood, where it can lead to efficient killing of bacteria.
- IgD remains membrane-bound and somehow regulates the cell's activation.
- IgE is found in trace amounts in the blood, but it still triggers allergies.

3. The biological effector functions of antibodies are mediated by the C domains.

4. Monoclonal antibodies are pure antibodies with single antigenic determinants.

- Monoclonal antibody development improves on nature.
- Special procedures are needed to screen for and isolate monoclonal antibodies.
- Monoclonal antibodies have many applications.

OBJECTIVES

The reader should be able to:

- Understand how the characterization of antibody structure led to the comprehension of antibody function.
- Distinguish between the overall structure and the fine structure of antibodies.
- Describe the variable and constant regions of an antibody's light and heavy chains.
- Explain the organization of the variable regions of an antibody's light and heavy chains; define hypervariable regions and domains.

- Name and compare the biological and chemical characteristics of the five classes of antibodies.
- Discuss the differences in the biological effector functions of antibodies.
- Contrast conventional antibody and monoclonal antibody development; conceptualize the procedure for monoclonal antibody screening; and discuss hybrid monoclonal antibodies.

In the preceding chapter, antigens were described.¹ This chapter describes the proteins that bind antigens and mark them for destruction by the immune system.

The recognition proteins found in the serum² and other body fluids of vertebrates that react specifically with the antigens that induced their formation are called **antibodies**. Antibodies belong to a family of globular proteins called **immunoglobulins**. The terms *antibody* and *immunoglobulin* are used interchangeably throughout this text. *Immunoglobulins*, however, are defined as a family of globular proteins that comprise antibody molecules and molecules having patterns of molecular structure (antigenic determinants) in common with antibodies. The term *immunoglobulin* can be used to refer to any antibody-like molecule, regardless of its antigen-binding specificity.³

THE STRUCTURE OF AN ANTIBODY IS RELATED TO ITS FUNCTION

The function of an antibody is to bind foreign or non-self molecules. The host can produce a vast array of antibodies that are structurally similar (all are Y-shaped molecules) yet unique. This variability was a startling finding because all other protein molecules made by an individual are identical; they all have the same amino acid sequence. However, antibodies come in millions of different amino acid sequences and are the most diverse proteins known.

The chemical structure of antibodies explains three functions of antibodies: (1) binding versatility, (2) binding specificity, and (3) biological activity. A mammal is capable of responding to more than 100 million antigenic determinants and can even respond to artificial antigens that do not exist in

FAST FOCUS 1

The estimation for an individual's antibody repertoire is calculated from theoretical assumptions. Nonetheless, the number is huge.

nature (Fast Focus 1). Because the amino acid sequence differs in the arms of various antibody molecules, each different antibody can bind specifically to

¹We use the term *antigen*, even though *immunogen* may be more accurate at times, as explained in Chapter 3.

²Antibody-containing serum is called *antiserum*, in contrast to *normal serum* (the clear yellowish fluid collected when whole blood is separated into its solid and liquid parts) that does not contain antibody to a specific antigen.

³MHC molecules and T-cell antigen receptors are the other two kinds of molecules used by the immune system to bind antigen. Their role in antigen recognition is discussed in Chapters 8 and 9, respectively.

one unique epitope. Thus, the arms of an antibody molecule confer the versatility and specificity of responses that a host can mount against antigens. The stem region of an antibody molecule decides its biological activity and defines whether the response against a particular antigen will lead to complement-mediated lysis, enhanced phagocytosis, or (in some cases) allergy. These activities start once antibodies bind to antigen.

Studies by Tiselius and Kabat and Later by Edelman, Porter, and Nisonoff Determined the Basic Structural Components of Antibodies

Initially, Antibodies Were Identified Only by Their Electrical Charge

Since the 1890s, immunologists have known that the molecules of humoral immunity are present in serum. In 1939, Tiselius and Kabat performed electrophoretic studies with rabbit antiserum specific for ovalbumin.⁴ They found that electrophoresis of serum from unimmunized rabbits resolved serum proteins into four dominant families of differing mobility. The albumins were the fastest-migrating (most negatively charged) fraction, followed by the alpha- (α), beta- (β), and gamma- (γ) globulins (Figure 4-1). Tiselius and Kabat then hyperimmunized rabbits with hen ovalbumin to get a strong antibody response. When they electrophoresed the immune serum, they observed a large increase in the γ -globulin fraction and concluded that antibodies were γ -globulins. After they absorbed out the anti-ovalbumin antibodies with the antigen ovalbumin, they observed that the electrophoretic pattern was the same as the pattern for the preimmune serum. This observation thus supported their conclusion that *antibodies are γ -globulins*. Antibodies can be separated further by ultracentrifugation. Studies performed in the 1940s and 1950s revealed that antibody molecules are heterogeneous in size. They range in molecular weight from 150 to 1000 kD.

Fragmentation Studies Showed That Antibodies Are Made Up of Two Identical Light Chains and Two Identical Heavy Chains

In the late 1950s and early 1960s, Rodney Porter of Great Britain and Gerald Edelman of the United States elucidated the chemical structure of antibodies.⁵ Edel-

⁴Electrophoresis is the migration of charged molecules after the introduction of an electric current.

⁵Porter and Edelman shared the 1972 Nobel Prize in Medicine for their structural studies of antibodies.

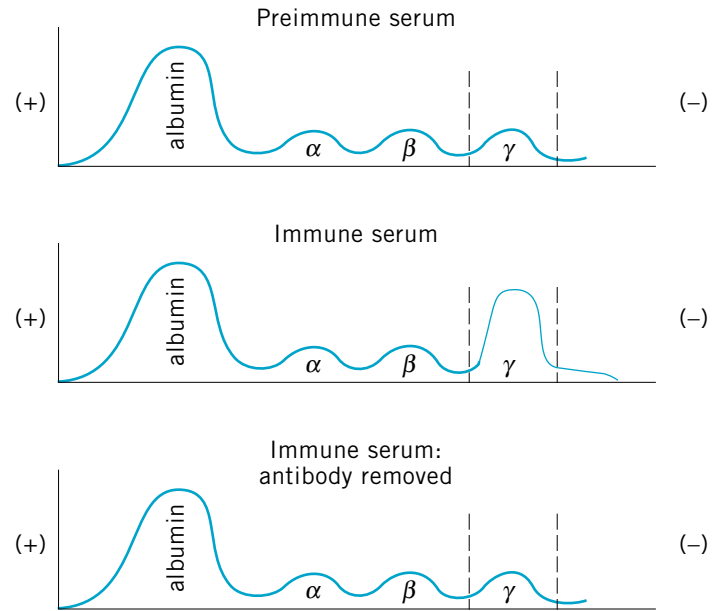


FIGURE 4-1 Antibody activity in the γ -globulin fraction of serum. The electrophoretic pattern of rabbit serum before immunization (top), after immunization (middle), and after absorption of the antibody with specific antigen.

man structurally studied γ -globulins and myeloma proteins, while Porter's experiments focused on a specific antibody from the γ -globulin fraction of rabbit serum called *immunoglobulin G* or *IgG*. Edelman's and Porter's approaches to the characterization of the IgG molecule were different. Edelman characterized IgG molecules using chemical solvents, whereas Porter used protein-degrading enzymes. Our understanding of antibody structure draws from the two scientists' results.

To generate subunits of IgG, Edelman treated rabbit IgG with dithiothreitol (a reducing agent that disrupts disulfide bonds), iodoacetamide (an alkylating agent that prevents reassociation of the disrupted disulfide bonds), and a denaturing agent (a substance that disrupts noncovalent interactions). When the treated antibodies were passed through sizing columns, he identified two subunits in equimolar ratios. He designated the larger subunit (50 kD) as the **heavy**, or **H, chain** and the smaller subunit (23 kD) as the **light**, or **L, chain**. Because the molecular weight of the original IgG molecule is 150 kD, he concluded that the IgG molecule consisted of two heavy and two light chains linked by disulfide bonds and noncovalent interactions.

Porter fragmented rabbit IgG with the proteolytic enzyme *papain* in the presence of the reducing agent cysteine. Papain hydrolyzed peptide bonds in IgG and produced three fragments (I, II, and III). The three fragments had similar molecular weights (50 kD) but different charges. Two of the three fragments were identical and retained the ability to bind antigen. These two fragments were called the **Fab fragments**

(for *fragments of antigen-binding*). Because the intact IgG is bivalent and the two Fab fragments each could bind antigen, Porter concluded that the Fab fragments must be univalent. The third fragment produced by papain digestion did not bind with antigen and crystallized during cold storage. Porter called this piece the **Fc fragment** (for *fragment crystallizable*). Thus the ratio of Fab to Fc is 2:1. Edelman confirmed Porter's results by cleaving and electrophoresing human IgG into two antigenically different fractions equivalent to the two fragments from rabbit IgG (Figure 4-2).

In similar studies, Alfred Nisonoff used *pepsin*, which hydrolyzes different sites on the IgG molecule than does papain. IgG treated with pepsin yielded one large fragment with a molecular weight (100 kD) double that of one Fab fragment, and many small fragments. Nisonoff called the large fragment **F(ab')₂**. This fragment also could bind antigen, but unlike the Fab fragment, it led to a visible serologic reaction. It had both of the antigen-binding sites of IgG (the chains remained linked) and could be treated further with reducing agents to yield two Fab-like fragments called **Fab'**. Collectively, the two enzymes cleave at about the same region of the IgG molecule. Papain splits the molecule on one side, and pepsin on the other side, of the bond that holds Fab fragments together (see Figure 4-2).

Following these studies, Porter showed that either Fab or Fab' fragments compose the entire light chain and part of the heavy chain. These data led to the formulation of the structure of an antibody and are summarized in Table 4-1. IgG is used as the prototype antibody to explain the basic structure of all antibodies.

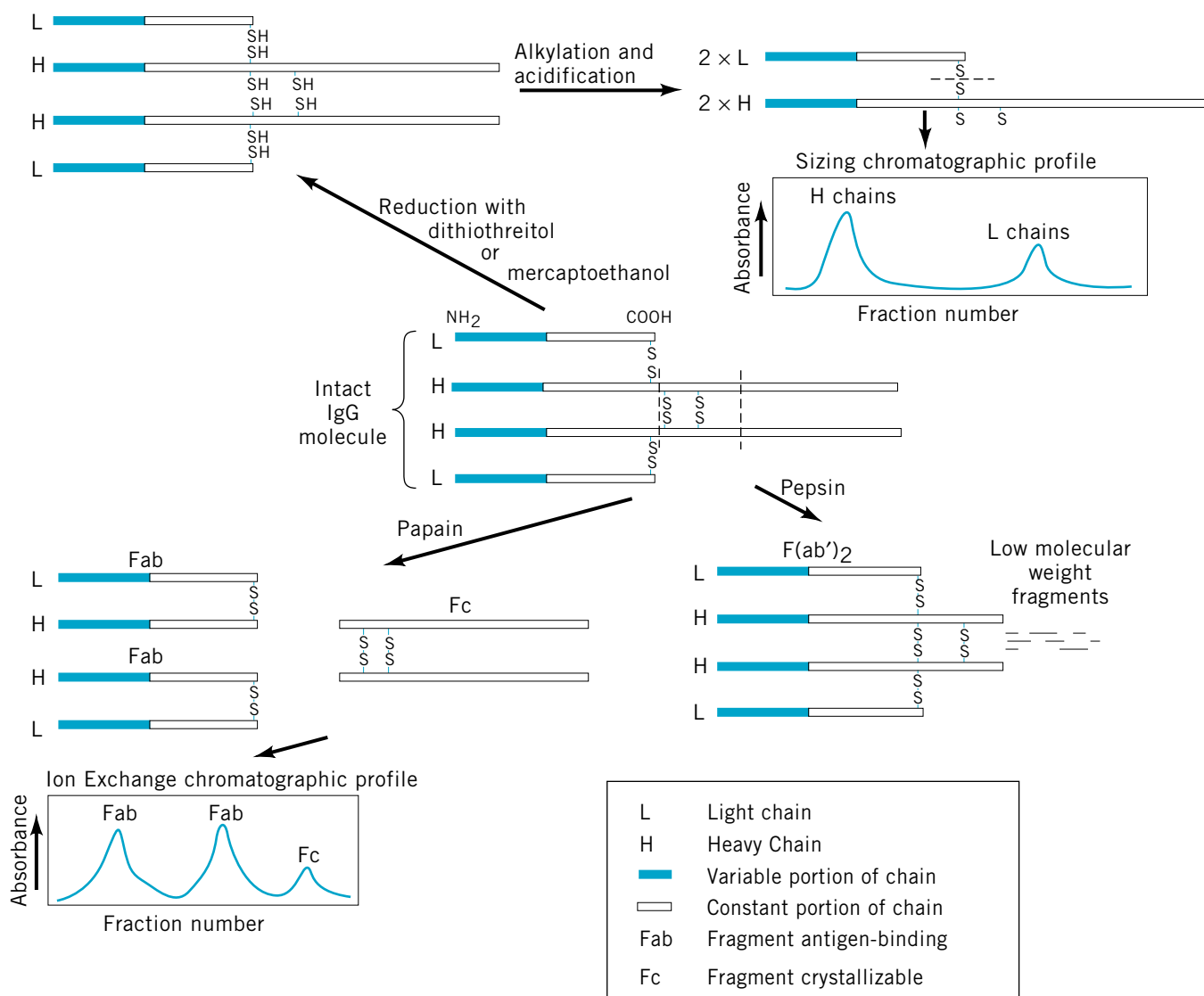


FIGURE 4-2 Fragmentation of rabbit IgG by the enzymes papain and pepsin.

Schematic illustration of the chromatography of reduced and papain-digested (also pepsin-digested) rabbit IgG. The results show the basic structure of all antibodies. See text for further explanation.

Further Studies Explained the Fine Structure of Antibodies—the Variable and Constant Regions on the Chains

The primary structure of antibodies was determined by amino acid sequencing of myeloma proteins. *Myeloma proteins* are molecules that have the characteristic structure of antibodies but originate from a disease state rather than from an immune response to a specific antigen. These pathologic immunoglobulins are formed by individuals with **multiple myeloma**, a cancer of antibody-forming (plasma) B cells. Myeloma proteins were invaluable in the resolution of antibody structure because they are produced in large quantities

and are usually homogeneous in a patient, whereas in a normal immune response the antibodies are heterogeneous in composition (**Fast Focus 2**). The main work involved a light-chain myeloma protein, called the *Bence-Jones protein* (first described in 1847 by Henry Bence-Jones). Bence-Jones proteins are immunoglobulin light

FAST FOCUS 2

Robert A. Good has described multiple myeloma as one of those “crucial experiments of nature,” a group of diseases that has “contributed maximally to the development of our concepts of immunology.” In popular parlance, the path of discovery went “from the patient’s bedside to the laboratory bench” and led to the complete mapping of an antibody’s structure. (R. A. Good, 1972. In *Clinical Immunology*, Vol. 1. New York: Academic Press, p. 23.)

TABLE 4-1 Mini Summary: Characteristics of the IgG Molecule*

- IgG (150 kD) consists of two light chains and two heavy chains; each light chain pairs with a heavy chain, and each heavy chain pairs with another heavy chain.
 - The chains are linked by covalent interchain disulfide bonds and noncovalent interactions.
 - The two light chains (23 kD each) and two heavy chains (50 kD each) are identical.
- Papain digestion produces two Fab fragments and one Fc fragment.
 - Fab fragments (50 kD) consist of the entire light chain and part of the heavy chain.
 - Fab fragments contain the antigen-binding sites.
 - The Fc fragment (50 kD) crystallizes in cold and does not bind antigen.
- Pepsin digestion produces one large fragment (100 kD) called $F(ab')_2$ and degrades the remainder of the heavy chains into small pieces.
 - When the large fragment is treated with reducing agents, the disulfide bridges are disrupted, giving two Fab-like fragments, Fab'.
 - Each of the Fab' fragments is made up of the entire light chain and a slightly longer part of the heavy chain.

*Even though characteristics are given for the IgG antibody molecule, we will see that all antibody molecules have similar overall structures and some common properties.

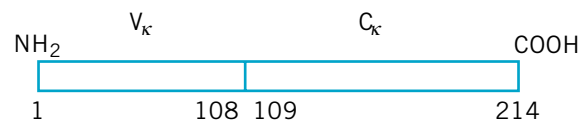
chains excreted in the urine of myeloma patients. Amino acid sequence comparisons of Bence-Jones κ light chains led to a startling finding. Each chain consisted of a large region that was constant in different types of antibodies (even from unrelated species) and a similar-sized region that was highly variable. This large region, called the **constant (C) region**, has amino acid sequences in the carboxyl terminal end (residues 109 to 214) of the κ chains that are almost identical. The opposite end of the κ chain, the amino terminal end (residues 1 to 108), shows great variability in amino acid sequence among the chains and is called the **variable (V) region**.

The amino acid sequence of antibody heavy chains showed similar regions. The first 113 amino acid residues of the heavy chain⁶ again showed great variability, while the remaining part of the heavy chain consisted of amino acids that were almost identical (the constant regions). This implies that molecules binding different determinants have different V light (V_L) and V heavy (V_H) regions. A group of words

⁶The length of the heavy-chain V region varies. The V heavy-chain region is somewhat larger (about 123 amino acid residues) than the V light-chain region (about 108 amino acid residues). V-region length depends on CDR length. The CDRs, especially the CDR3s of IgMs, can be quite large.

FIGURE 4-3 Amino acid sequence of light and heavy chains—localization of variability. Antibodies have two identical light and heavy chains. Each chain is divided into two regions, the variable (V) and constant (C) regions. The amino-terminal end contains the V region, while the carboxyl-terminal end contains the C region. In the V region are areas of increased variability called *hypervariable regions* or *complementarity-determining regions* (CDRs, marked by arrows); the numbers below the arrows give the amino acid residue positions. The CDRs of the light and heavy chains form the antigen-binding sites. The more conserved amino acids between the CDRs are called *framework residues*; they hold the CDRs in place. See text for further explanation.

Light chains (κ chains)



Heavy chains ($\gamma 1$ chains)



Location of CDRs in V_κ region



Location of CDRs in V_H region



FAST FOCUS 3

Antibodies are large proteins shaped to form a Y. The sections that make up the tips of the Y's arms, or variable (V) regions, vary greatly from one antibody to another, creating a pocket uniquely shaped to enfold a specific antigen epitope. The fork in the Y is the hinge region, which connects the arms to the stem and allows them to swing. The stem, or constant (C) region, of the Y serves to link the antibody to other participants in the immune response. The stem is identical in all antibodies of the same class.

can illustrate the concept: *P-S-Y-C-H-O-L-O-G-Y*, *P-H-Y-S-I-O-L-O-G-Y*, and *I-M-M-U-N-O-L-O-G-Y*. All have a constant region ("O-L-O-G-Y") and a variable region, the first five letters of each word (Fast Focus 3). Because amino acid sequence dictates the three-dimensional structure of protein, the unique sequence of amino acid residues for each V region leads to the large diversity of structure,

which accounts for antibody specificity.

Variability of the amino acid sequences in the V regions is not random but precisely organized. It is localized within certain sections of the V region of a chain, and these sections have substantial sequence variation from protein to protein. The greatest variability in the light chain is around residues 30, 55, and 95. These

areas are called **hypervariable regions** or **complementarity-determining regions (CDRs)**. Moving from the amino-terminal end of an antibody chain, the three regions are called CDR1, CDR2, and CDR3. Each CDR is about 10 amino acid residues in length. (The V region of the heavy chain has three CDRs near the CDRs of the light chain.) Hypervariable regions are called CDRs because these segments line the antibody-combining site. Intervening sequences between the CDRs have restricted variability and show little difference in amino acid sequence between chains. These invariant segments make up the **framework residues**, which compose about 85% of the V region. Framework residues define the positioning of the CDRs. The V region folds so that the CDRs are exposed on the surface of the chain. When the light and heavy chains are joined, the CDRs of the chains form a cleft that serves as the antigen-binding site of an immunoglobulin. Because the amino acid sequences of the CDRs determine the shape and ionic properties of the antigen-binding site, the CDRs define *the specificity of the antibody*. A schematic representation of heavy and light chains is shown in Figure 4-3.

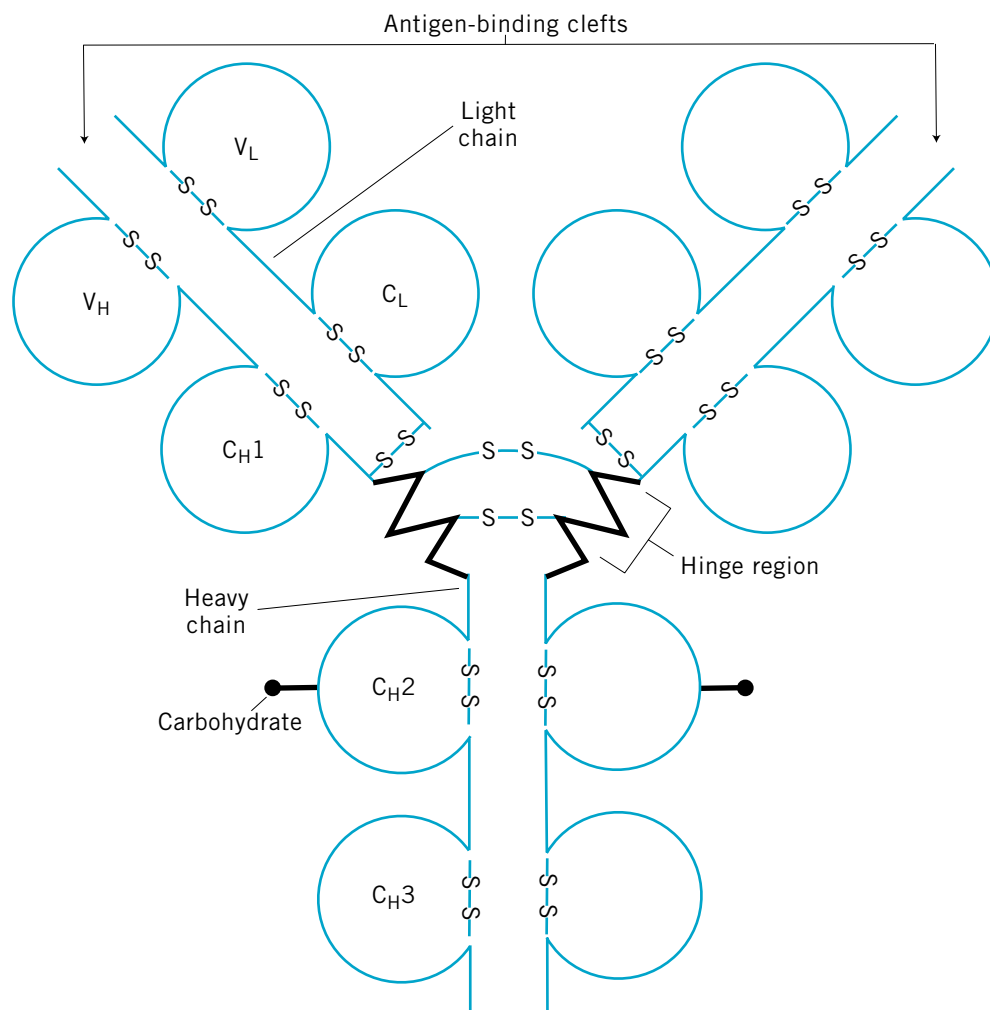


FIGURE 4-4 Schematic representation of IgG domains.

Areas of variability and constancy, divided into segments of 110 amino acid residues, within an IgG molecule (and for all antibodies) are known as *domains*. The IgG molecule has a V and a C domain for each light chain and one V and three C domains for each heavy chain.

Although antibodies are complex globular proteins, they do possess some structural repetitiveness. Edelman showed that *each antibody chain has a tandem series of repeating homology units roughly 110 amino acid residues in length called immunoglobulin domains, which fold independently into a compact globular structure* (Figure 4-4, see page 63). All proteins that exhibit this structural motif belong to the **immunoglobulin gene superfamily** (see Chapter 9). Domains are separated by less ordered regions of the peptide chains. The light chain of IgG has two domains called V_L and C_L . The heavy chain of IgG has four domains: one V_H and three in the C_H region (C_{H1} , C_{H2} ,

and C_{H3} or $C_{\gamma 1}$, $C_{\gamma 2}$, and $C_{\gamma 3}$). The heavy-chain V unit shows similarity to the V part of the light chain, while the three C-region units show strong homology to each other and to the C region of the light chain. Each domain has a characteristic tertiary structure consisting of two β -pleated sheet structures. Each domain has a sandwich-like structure with a hydrophobic interior. Layers are covalently linked by a disulfide bridge near the center of the domain. When the V_L chain framework residues assume this three-dimensional arrangement, loops are held out from the sandwich. These loops are the CDRs. The size and shape of the loops are defined by their amino acid residues. When heavy and

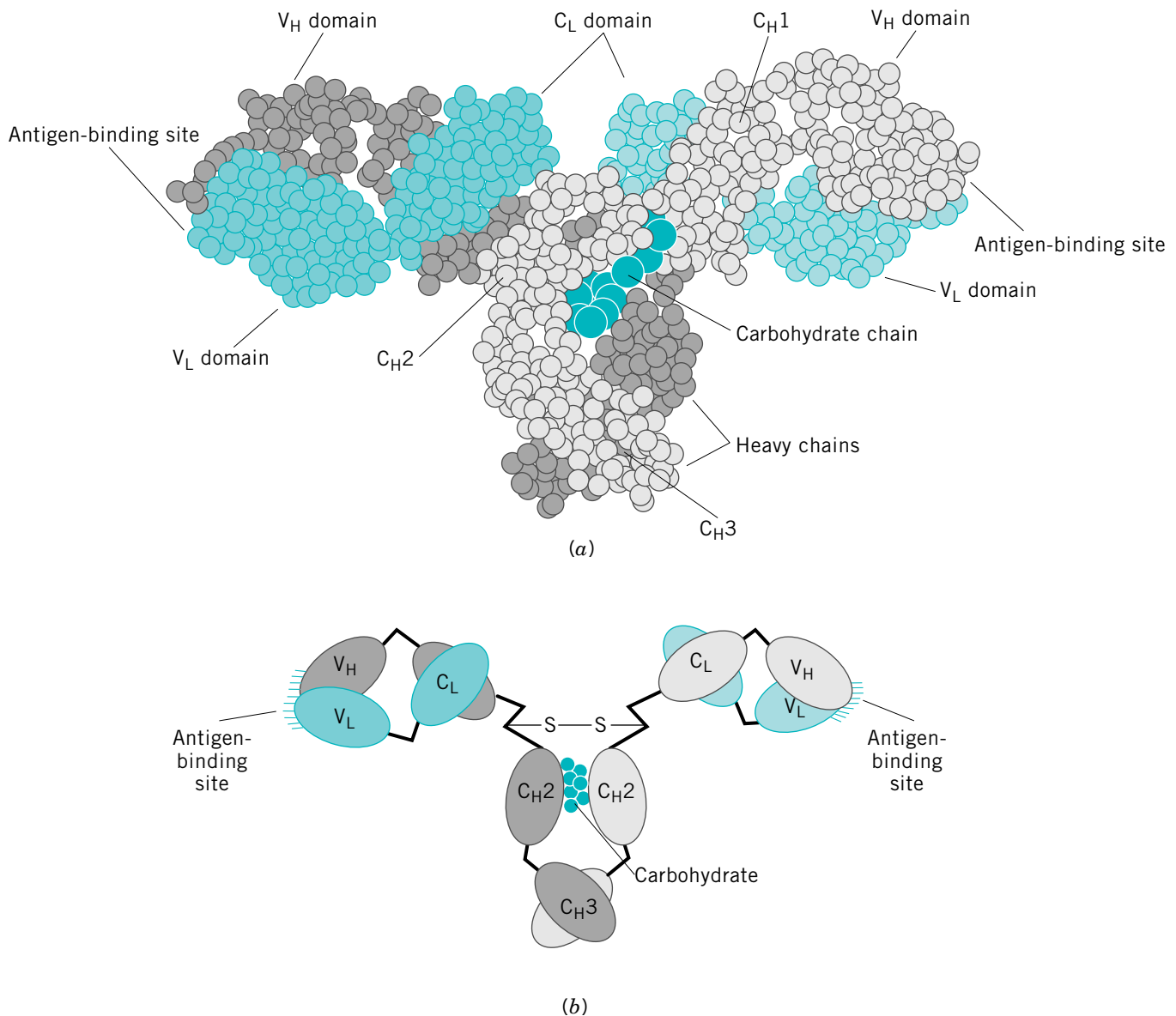


FIGURE 4-5 Three-dimensional model of IgG. From studies by X-ray crystallography and electron microscopy, (a) the three-dimensional structure of IgG can be depicted as a Y-shaped molecule. Each sphere represents an amino acid residue. The two light chains are shades of blue and the two heavy chains are shades of gray. (b) The schematic cartoon shows how the domains interact. (Adapted from E.W. Silverton, M.A. Navia, and D.R. Davies. 1977. *Proc. Natl. Acad. Sci. USA* 74:5140.)

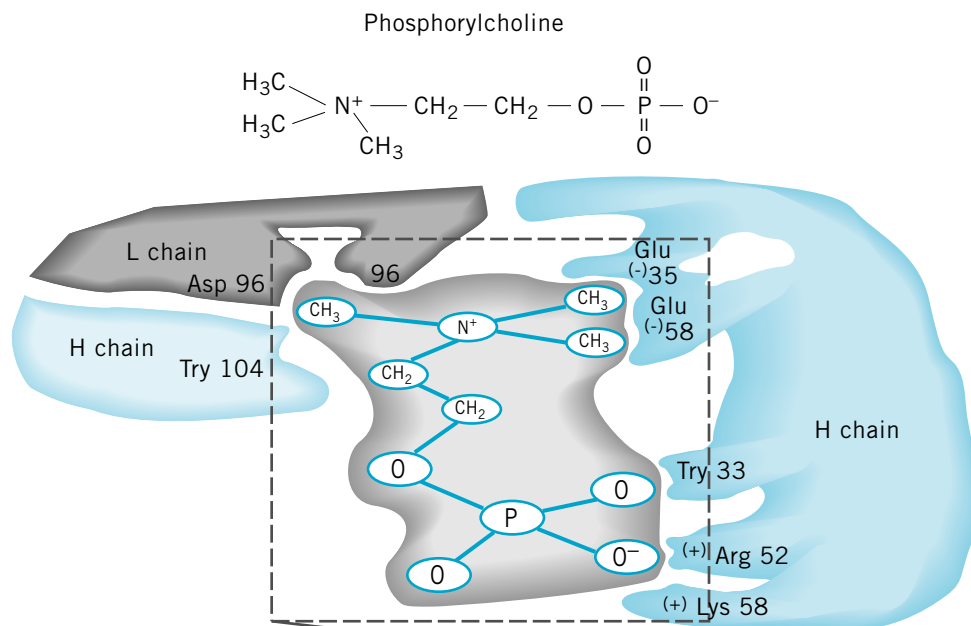
light chains are brought together to form an IgG molecule, extensive noncovalent interactions occur between V_L and V_H , C_L and C_{H1} , C_{H2} and C_{H2} , and C_{H3} and C_{H3} . These regions of interaction are shown by the “three-dimensional” model in Figure 4-5 (see page 64). The association of the CDRs and the antigen-binding site of an antibody was confirmed by crystallographic studies. An example of such a study is the binding of antibody specific for the hapten phosphorylcholine (Figure 4-6).

Antibodies also demonstrate **segmental flexibility**, which means that the two Fab portions can move relative to one another on antigen binding. The angle varies from 60 to 180 degrees. This flexible region where the arms meet the stem of the Y is called the **hinge region** and is located between the C_{H1} and C_{H2} domains. Only IgG, IgA, and IgD antibody molecules⁷ have hinge regions. The heavy chains of IgM, IgA, and IgD antibody molecules possess additional amino acid residues on the carboxyl-terminal end of the last C_H domain. These areas, called **tail pieces**, permit IgM and IgA to interact with like antibodies and form multimeric molecules. Multimeric IgM and IgA also have a polypeptide called the **joining (J) chain**, which is disulfide-linked to the tail pieces and stabilizes the multimeric structure. Antibodies also contain carbohydrates; the percentage and location of the carbohydrate molecules differ according to the class of antibody.

⁷The five antibody classes—IgG, IgA, IgM, IgD, and IgE—will be discussed shortly.

MINI SUMMARY

Amino acid sequencing of light chains shows that variability is localized at the amino terminal end of the chains in the variable, or V, regions. The other (carboxyl) ends are identical and are called the constant, or C, regions. This variability arrangement is the same for heavy chains. Variability is concentrated in segments called complementarity-determining regions (CDRs) or hypervariable regions. The CDRs line the walls of the antigen-binding sites in antibody molecules. Segments between the CDRs are highly conserved regions called framework residues. Light chains also possess two repeated homology units (domains), and heavy chains possess four to five domains. The three-dimensional structure of antibodies shows that domains are folded in similar configurations, with the V-light and V-heavy units lying together to form the V domains of the complete antibody molecule. V domains contain the antigen-binding cleft. Changes in amino acid residues at the position of this cavity change its shape and thus its specificity. The amino acid sequencing of antibody light and heavy chains divides them into V regions for antigen recognition and C regions for biological effector functions.



The cleft formed by the amino acids of the heavy (H) and light (L) chain complementary determining region (CDR) “holds” the antigenic determinant, in this case the hapten phosphorylcholine.

FIGURE 4-6 Antigen-combining site of an antibody for the hapten phosphorylcholine showing the complementarity between an epitope and an antibody-combining site. The amino acids lining the CDRs bind to the epitope through weak, noncovalent interactions. The three CDRs of the heavy chain and one CDR of the light chain contribute to the combining site.

The Antibody's Antigenic Determinants—Called Isotypes, Allotypes, and Idiotypes—Determine the Variability in Antibody Structure

Humans express five groups of antibodies, called **immunoglobulin (Ig), or antibody, classes**. The

five classes of antibodies, designated *IgG*, *IgA*, *IgM*, *IgD*, and *IgE*, differ in their physicochemical (charge, size, and solubility) and serologic (*in vitro* reactions with antigens) properties, and *in their behavior as antigens*. The latter characteristic usually is the one used to divide immunoglobulins into the five classes.

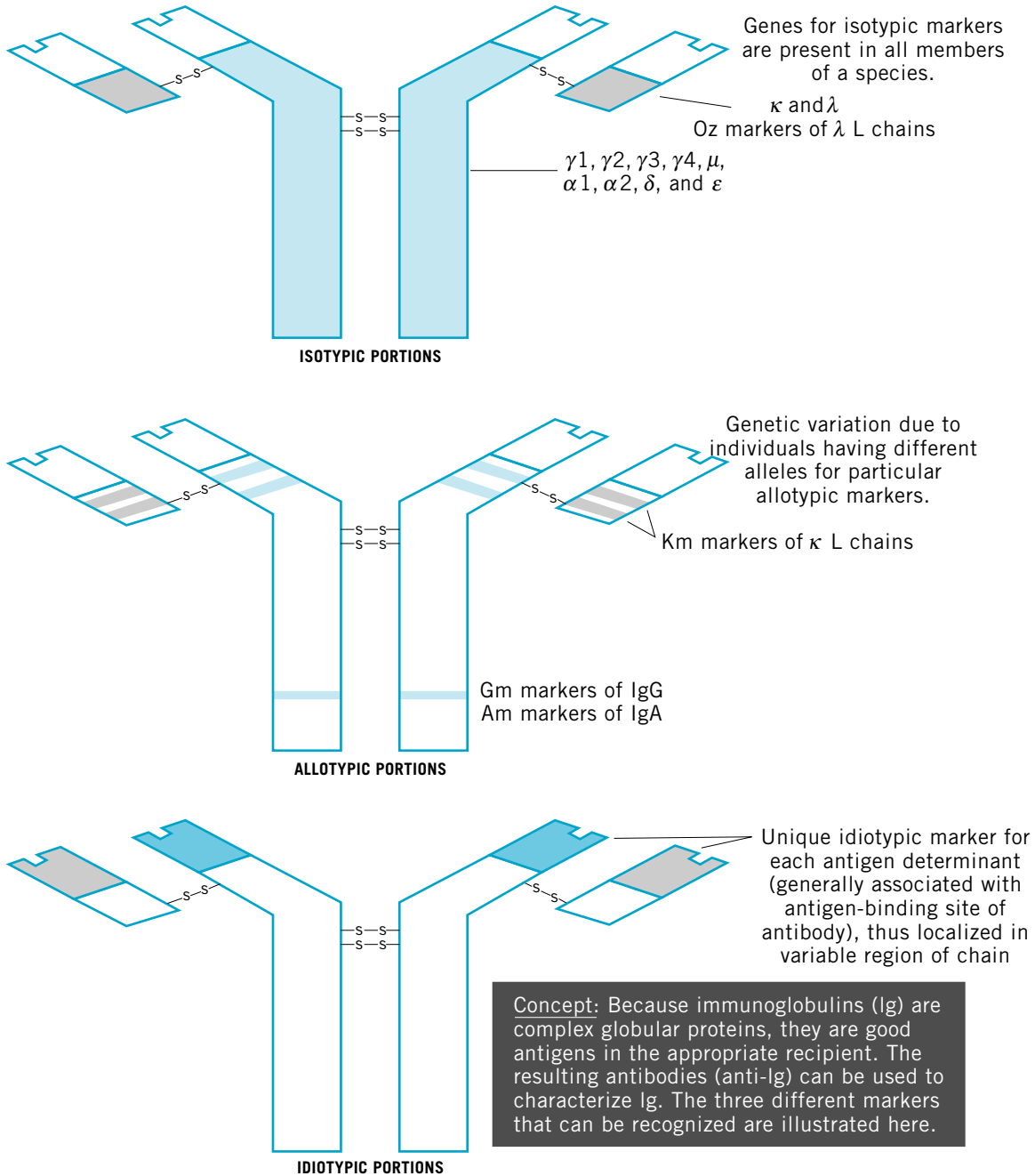


FIGURE 4-7 Nomenclature of antibody variability. Three overall variants of antibody exist. Going from general to specific variation, the first, known as *isotypic variation*, refers to the different heavy and light chain classes (IgG, IgA, IgM, IgD, and IgE) and subclasses (such as human IgG1 through IgG4). Most *allotypic variation* occurs in the C region of H and L chains, but some has been found in the framework residues of V regions. The last variation, called *idiotypic*, is found only in the V regions of the heavy and light chains.

Antibodies are divided into classes by the antigenic determinants on their heavy chains. Antibodies themselves can be immunogenic. For example, if one immunizes a rabbit with human antibodies, the rabbit's immune system does not see an antibody molecule but rather a foreign complex glycoprotein. The rabbit responds by making antibodies to the human antibodies, and this antiserum can be used to distinguish at least three types of determinants (*isotypic*, *allotypic*, and *idiotypic*) that can then be used to classify antibodies into *isotypes*, *allotypes*, and *idiotypes* (Figure 4-7).

Isotypes Are Variants Present in All Members of a Species

The heavy-chain antigenic determinants that define the antibody class are called **isotypic determinants**. The antibody molecule is an **isotype** (G. *iso*, the same). *Isotypic determinants distinguish C-region sites*. Thus, an antibody specific for the heavy chain of IgG reacts with only IgG molecules. Antigenic determinants that define the heavy chain of IgG, IgA, IgM, IgD, and IgE are given the Greek letters gamma (γ), alpha (α), mu (μ), delta (δ), and epsilon (ϵ), respectively. Other isotypic heavy-chain determinants define differences within a class and thus are called **antibody subclasses**. IgG has four subclasses, called $\gamma 1$, $\gamma 2$, $\gamma 3$, and $\gamma 4$ or IgG1, IgG2, IgG3, and IgG4. In humans, IgG1, IgG2, IgG3, and IgG4 are found in normal serum in the approximate proportions of 65, 25, 5, and 5%, respectively. Two subclasses have also been found in IgA, designated $\alpha 1$ and $\alpha 2$ or IgA1 and IgA2.

Antigenic determinants on light chains classify them as either κ or λ chains. Humans have only one gene for the κ C region, but the λ chain has at least four functional C-region genes. The four λ chain isotypes are designated $\lambda 1$, $\lambda 2$, $\lambda 3$, and $\lambda 6$. Light-chain antigenic determinants are *not* useful in determining antibody class or subclass because κ and λ chains are associated with all classes and subclasses. Within an antibody molecule, both heavy chains and both light chains are identical. All of the genes for isotypic determinants are expressed in a normal individual; therefore, all isotypic determinants are present on some antibodies of all members of a species.

Allotypes Are Variants Caused by Intraspecies Genetic Differences

Allotypic determinants are encoded by one allele (variation) of a given antibody gene and are present on the antibodies of some members of a species. These antibodies are called **allotypes** (G. *allos*, other). Alternate alleles code for antigenically distinct allotypic markers. The genes encoding allotypic determinants are inherited in a Mendelian fashion and illustrates the genetic diversity within a species (*polymorphism*). Allotypic nomenclature consists of the right class and

subclass marker followed by each allele in parentheses—for example, G2m(23), G4m(4a), A2m(1), or Km(3). The γ , α , and κ chains have allotypic determinants called **Gm**, **Am**, and **Km**. All four γ chain isotypes have allotypes (humans have more than 20 Gm markers). IgA2 has allotypes designated A2m(1) and A2m(2). The κ -light chain allotypes are called Km(1), Km(1,2), and Km(3). No allotypes have been described for IgM, IgA1, IgD, IgE, or the λ chain.

Idiotypes Are Variants Caused by Structural Heterogeneity in the Antibody V Regions

Idiotypic determinants are found in the V region near the antigen-binding site of the antibody molecule. These determinants classify antibodies into **idiotypes** (G. *idios*, one's own) and are common to antibodies having specificity for the same foreign antigenic determinant. Each individual has as many different idiotypes as it has different antibodies. These determinants are individual-specific. Idiotypic determinants reflect the antibody-combining site's structure and usually depend on the arrangement of heavy and light chains.

MINI SUMMARY

The antigenic determinants of antibodies show three levels of variability. The determinants (from general to specific) are called isotypic, allotypic, or idiotypic and classify antibody molecules into isotypes, allotypes, and idiotypes, respectively. Isotypic determinants, found on the C-region part of heavy chains, divide antibodies into five classes (or isotypes) called IgG, IgA, IgM, IgD, and IgE. Isotypic determinants also can be used to classify antibody light chains into κ and λ chains. Isotypic differences can be used to partition classes into subclasses. Allotypic determinants are carried by only some individuals within a given species and are inherited in a Mendelian fashion. Idiotypic determinants are individual-specific and represent the antigen-combining site of an antibody.

THERE ARE FIVE CLASSES OF ANTIBODIES BASED ON THE STRUCTURE OF THEIR HEAVY-CHAIN C DOMAINS

All Antibody Classes Have Either λ or κ Light Chains

As mentioned earlier, the two determinants used to define antibody light chains are called kappa (κ) and lambda (λ). Each chain has a molecular weight of about 23 kD and exists as one polypeptide chain of about 214 amino acid residues. The first 108 amino acids make up the V region, followed by roughly 110

amino acids that make up the C region. These two regions compose the V_L and C_L chain domains. The ratios of κ to λ light chains varies greatly within mammalian species. In mice, it is 95:5; in humans, it is about 60:40. However, there is no difference in either chain's ability to pair with a heavy chain.

IgG Is the Major Antibody in the Blood, but It Is Able to Enter Tissue Spaces and Coat Antigens, Speeding Antigen Uptake

IgG, primarily induced by protein antigens, constitutes about 80% (12.5 mg/ml) of the antibody in serum. The IgG (150 kD) is composed of two light chains (either κ or λ) and two heavy chains (γ). The four polypeptide chains are covalently held together by disulfide bonds. Human IgG consists of four subclasses (isotypes), which are numbered in order of their serum concentrations (IgG1, IgG2, IgG3, and IgG4). The four subclasses have 90 to 95% identity with each other in the C-region domains. The γ chain is made up of four domains, one in the V portion and three in the C portion of the chain. The γ_1 chain is the shortest heavy chain, with 446 amino acid residues. On the C_{H2} domain (at position 297) of all γ chains is attached one carbohydrate group that controls the quaternary structure of this domain. The chief distinguishing characteristic among the four IgG subclasses is the pattern of interchain linkages in the hinge region.

IgA Concentrates in Body Fluids to Guard the Entrances of the Body

Human IgA constitutes only 13% (2.1 mg/ml) of the antibody in human serum, but it is the predominant class of antibody in extravascular secretions. The IgA present in secretions (tears, saliva, nasal secretions,

bronchial and digestive tract mucus, and mammary gland secretions) is **secretory IgA**. While the precise organization of secretory IgA is unknown, the model of current choice is depicted in Figure 4-8.

The *J chain* is a 15-kD polypeptide consisting of 129 amino acid residues and one carbohydrate group. It is synthesized by plasma cells and attaches to IgA (or IgM) either before or at the time of secretion. The J chain attaches to the carboxyl-terminal penultimate cysteine of either the α or the μ chain. Dimeric IgA binds to the blood side of the epithelial cells through Fc receptors (Figure 4-9). These receptors are also called *secretory*, or *S*, *proteins*. Bound IgA is internalized and moves through the cytoplasm of the epithelial cells. IgA is detached from the cell following cleavage of S protein. The remaining peptide, called *secretory component* or *piece*, attaches to dimeric IgA. Depending on the species, it may or may not be disulfide-linked to the IgA dimer. It also gives resistance to enzymatic cleavage while in mucosal secretions.

The α chain is made up of one V domain and three C domains. IgA1 is the most prevalent form in serum, but IgA2 is slightly more prevalent in secretions. Only IgA2 has allotypic determinants, and only the A2m(1) uniquely lacks interchain disulfide bridges between light and heavy chains. Instead, chains are linked to their own counterparts (one light chain to the other light chain). Another difference between IgA allotypes is the size of their hinge regions.

IgM Is the Largest Antibody; It Tends to Remain in the Blood, Where It Can Lead to Efficient Killing of Bacteria

IgM, primarily induced by polysaccharide antigens, is a 950-kD pentamer that makes up about 8% (1.25

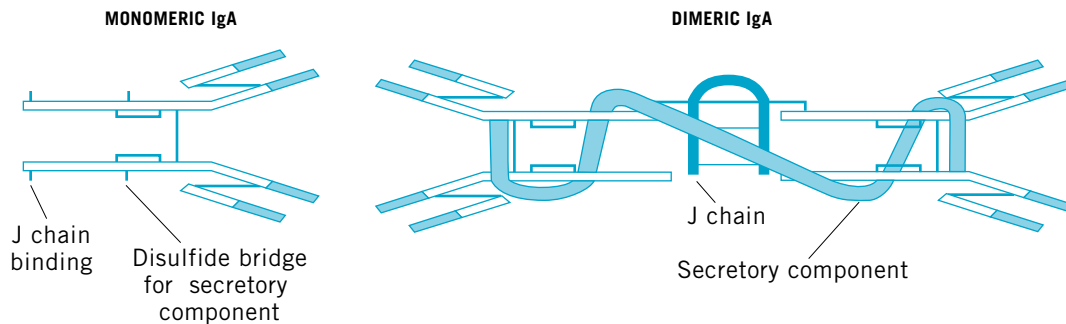


FIGURE 4-8 Human secretory IgA. This is made up of two monomeric IgA molecules, a joining (J) molecule and a secretory component (SC), with molecular weights of 160,000 (each monomer), 15,000, and 75,000, respectively. Intact secretory IgA has a molecular weight of 390,000. The two monomers of IgA are joined by their Fc ends by the J chain. They are attached by disulfide bridges to the $C_{\alpha 2}$ domain of each IgA monomer. The secretory piece is probably wrapped around the two IgA monomers.

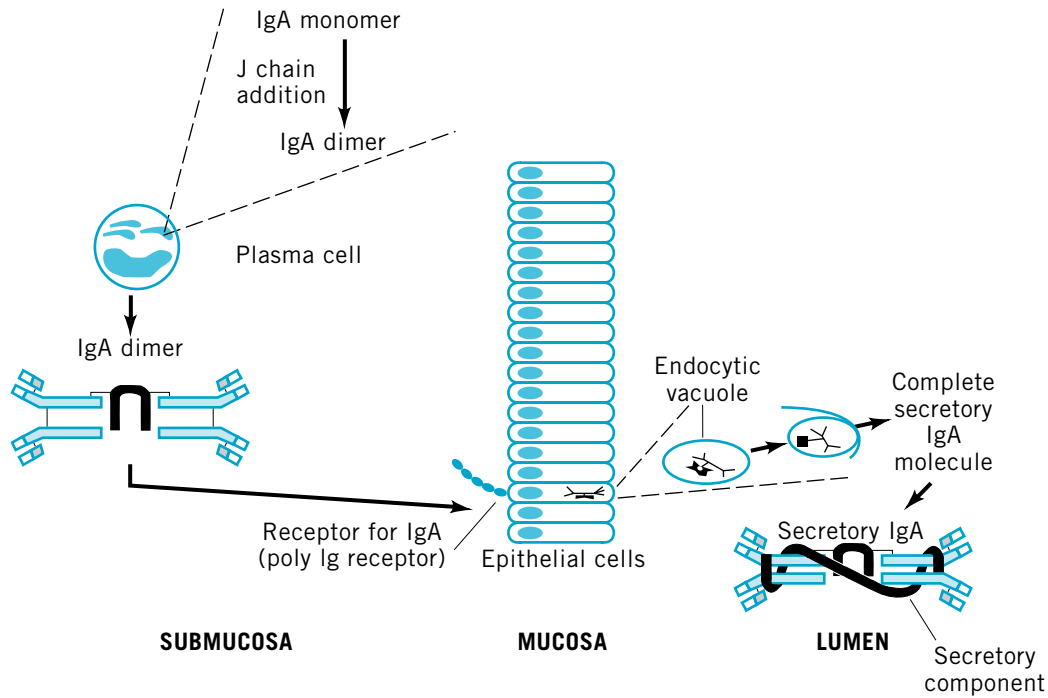


FIGURE 4-9 Synthesis and transport of secretory IgA. IgA molecules are synthesized as monomers, but plasma cells secrete them as dimers linked by the J chain. These IgA molecules bind to the poly immunoglobulin receptor on the interior surface of the epithelial cells. The complex is endocytosed and passed through the epithelial cells lining the lumens of the body, where the complex acquires the secretory component by cleavage of the receptor and finally enters the lumen as secretory IgA.

mg/ml) of the antibody in the serum. The five monomeric IgM molecules are arranged radially, the Fab fragments pointing outward and the Fc fragments pointing to the center of the circle (Figure 4-10). IgM is the first antibody to appear during an immune response and the first formed by a developing fetus. Because of its many antigen-binding sites, IgM can quickly clump antigen and efficiently activate complement. IgM acts as one of the main receptors on the surface of mature B cells, along with IgD. When IgM is a surface receptor, it is in its monomeric form.

The IgM μ chain consists of 576 amino acid residues, with 452 making up the C region. Unlike γ and α chains, which have three C-region domains, the μ chain has four. The five carbohydrate groups are in the C_{H1} and C_{H3} domains and in the part of the μ chain where the J chain binds. The C_{H2} domain of the μ chain is equivalent to the hinge regions of the γ and α chains. The μ chain has two interchain disulfide bonds. The membrane form of IgM has a different carboxyl-terminal end. The membrane form of IgM is made up of 41 additional amino acid residues, of which 25 form a transmembrane segment of hydrophobic (nonpolar) amino acids followed by hydrophilic (polar) amino acids.

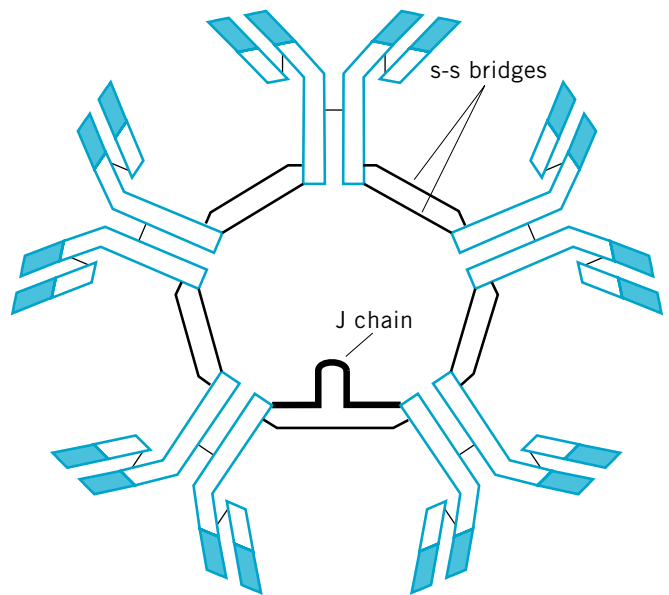


FIGURE 4-10 Human IgM. This is a pentameric (five monomeric IgM molecules) polypeptide chain that has four domains in each of the heavy chains. Disulfide bonds cross-link adjacent $C_{\mu 3}$ and $C_{\mu 4}$ domains of individual monomeric molecules. The possible location of the J chain is given.

IgD Remains Membrane-Bound and Somehow Regulates the Cell's Activation

IgD (175 kD) constitutes less than 1% (40 $\mu\text{g/ml}$) of the antibody in human serum. IgD is an antibody whose function remains unknown, even though it is one of the main receptors on mature B cells. As B cells mature, IgD is replaced by other antibodies. IgD may be a regulator of immune responses through its role in antigen internalization. The δ -chain C region is divided into three domains and consists of 383 amino acid residues. The hinge region of IgD consists of 64 amino acid residues, longer than any other antibody class.

IgE Is Found in Trace Amounts in the Blood, but It Still Triggers Allergies

Human IgE (190 kD) makes up less than 0.003% (0.4 $\mu\text{g/ml}$) of the antibody in serum. IgE binds through its Fc part to mast cells or basophils. On later exposure to the same antigen, mast cells and basophils bind antigen with membrane-bound IgE and trigger allergic reactions. IgE protects against parasites by releasing mediators that attract eosinophils. Like the μ chain, the ϵ chain contains four C-region domains. IgE is made up of about 13% carbohydrate. The ϵ chains are similar in size to μ chains, except that ϵ chains lack the 18 amino acid residues for J-chain binding. For further discussion of IgE, see Chapter 15.

THE BIOLOGICAL EFFECTOR FUNCTIONS OF ANTIBODIES ARE MEDIATED BY THE C DOMAINS

Whereas a small part of the V region on an antibody determines the antigen specificity, single domains in the C region of heavy chains determine the effector functions. *Biologic activities of antibodies divide into three general areas: (1) protection, (2) placental transfer, and (3) cytophilic (literally, "cell-loving") properties.* (The biologic function of membrane antibody as the B-cell receptor for antigen is discussed in Chapter

6.) Although antibody binding blocks the attachment of toxins or viruses (called *neutralization*), antibodies alone cannot directly destroy a foreign organism. Instead, antibodies mark them for destruction by other defense systems. When IgM or IgG (except IgG4) binds to antigen, the complement system is activated and promotes bacterial lysis or accelerated phagocytic uptake. IgM or IgG molecules that have not reacted with antigen do not activate complement. IgM also mediates agglutination reactions. The coating (*opsonization*) of organisms with primarily IgG antibodies leads to enhanced phagocytosis by macrophages and neutrophils. Antibodies allow for the interaction of several cell types with antigen–antibody complexes through the cells' Fc receptors. Three groups of human Fc receptors for IgG have been described (Table 4-2). Because of their characteristic immunoglobulin-like extracellular domains, Fc receptors belong to the immunoglobulin gene superfamily (see Figure 9-4 in Chapter 9). Multiple biologic functions can be triggered through the crosslinking of any of the three Fc receptor classes. Macrophages have enhanced engulfment of antigen–antibody complexes through Fc receptors. B-cell Fc receptor engagement by antigen–antibody complexes regulates B-cell activation. Other cell types expressing Fc receptors (CD16) can use antibody-dependent cell-mediated cytotoxicity (ADCC), to lyse target cells coated with IgG. Certain antibodies, like IgA, can be localized to the lumens of mucosal organs to provide mucosal immunity.

The second area of biologic activity associated with the antibodies is the movement of maternal antibody across the placenta to the fetus. The human fetus and newborns have limited immune responses. Mechanisms of acquired immunity are not at full strength until some time after birth. Most of the protection for a fetus or newborn comes from maternal IgG that crosses the placenta during pregnancy. Only IgG can cross the placenta because only the γ -chain C_{H1} and C_{H3} domains can bind to placental cells. Intact IgG or Fc fragments from IgG can cross the placenta, but Fab or $F(ab')_2$ cannot. Maternal IgA, secreted in breast milk, neutralizes pathogens in the infant's gut.

TABLE 4-2 Human IgG Fc Receptors

CD Name	Common Name	Molecular weight ($\times 1000$)	Affinity (K_d)	Distribution
CD16	FcRIII	50–65	2×10^{-6} M	Granulocytes, macrophages, NK cells, neutrophils
CD32	FcRII	40	5×10^{-7} M	B cells, eosinophils, granulocytes, macrophages
CD64	FcRI	75	1×10^{-8} M	Monocytes, macrophages, cytokine-activated neutrophils

Note: Three classes of human Fc receptor for IgG have been defined. The high-affinity FcRI is constitutively expressed on monocytes, macrophages, and cytokine-activated neutrophils, while the lower-affinity FcRII is widely distributed (found on immune and nonimmune cells). FcRIII has the lowest affinity of the three Fc receptors and is the main Fc receptor on neutrophils.

The third area of biologic activity is the binding of IgE to mast cells and basophil receptors through their Fc regions. Because of this “stickiness,” IgE antibodies are called *cytophilic antibodies*. The reaction following the second exposure of specific antigen with IgE molecules bound to mast cells triggers allergic responses. The IgE molecule and allergic responses are detailed in Chapter 15. Lymphocytes have Fc receptors for IgG that regulate IgG–antigen complex-mediated antibody feedback (see Chapter 12).

MINI SUMMARY

Antibodies are divided, based on their heavy chain structure, into six chemically distinct classes—four kinds of IgG and two kinds of IgA, plus IgM, IgD, and IgE. Each class plays a different role in the immune system’s defense strategy. IgG, the major antibody in the blood, coats microorganisms to speed up their uptake by phagocytic cells. The dimeric IgA concentrates in body fluids to guard the entrances of the body. IgM, a pentamer, is the most effective activator of complement (and therefore, is the best indirect killer of blood-borne bacteria). IgD is found almost exclusively on the surface of B cells, where it may regulate the cell’s activation. IgE, found in trace amounts in the blood, attaches itself to specialized cells, where it triggers the symptoms of allergies.

Antibodies have many biologic activities. They can prevent toxins and viruses from entering cells, whereas other antibodies coat bacteria or target cells to make them more palatable or vulnerable to attack by certain immune cells. Antigen–antibody complexes also can activate complement. Some antibodies, like IgG, cross the placenta and provide protection to the fetus. IgE can promote allergies. IgG-containing antigen–antibody complexes attach to Fc receptors on B cells, and inhibit B-cell activation.

MONOCLONAL ANTIBODIES ARE PURE ANTIBODIES WITH SINGLE ANTIGENIC DETERMINANT SPECIFICITIES

Normal serum contains 10^{16} antibody molecules per milliliter. These antibodies can be collected from experimental animals and have long been an important tool of investigators, who have used them to identify or label molecules or cells and to separate molecules or cells from mixtures. A concern, however, has always been the variability of antisera (Figure 4-11, see page 72). The development of hybridization techniques allowed

for the production of one kind of *specific* antibody by immortalized antibody-producing cells. Methods were then developed to screen for and produce large numbers of these antibodies for use in many applications.

Antibodies of a single idiotype produced by immortalized B cells are called **monoclonal antibodies**. Regrettably, normal antibody-secreting cells are end cells of a differentiation series; thus they cannot be maintained in culture. In contrast, myeloma cells are immortal. Therefore: “Why not use immunoglobulins produced by myeloma cells?” The reason myeloma immunoglobulins cannot be used is twofold: (1) their antigen specificity is usually unknown and (2) it is difficult to tailor-make antigen-specific myelomas. The second problem is overshadowed by the question “Is the combining site of the myeloma protein an accurate representation of the antibody produced during an immune response?” What would happen if one combined the characteristics of each cell into one? Georges Kohler and Cesar Milstein fused cells secreting antibody of one specificity with myeloma cells. The resulting clone of cells is a *hybrid-myeloma* or a **hybridoma**. The hybridoma cells inherit the lymphocyte’s property of specific-antibody production and the immortality of the myeloma cell. In 1975, Kohler and Milstein published a short paper in *Nature* detailing how continuous cultures of fused cells secreting a monoclonal antibody of predefined specificity were produced. They were awarded the 1984 Nobel Prize in Physiology and Medicine for their work.

Monoclonal Antibody Development Improves on Nature

How are hybridomas produced? Mice are immunized with specific antigens (Figure 4-12, see page 73), and spleens from hosts with the highest titers are collected. The separated spleen cells are mixed with myeloma cells that cannot produce immunoglobulins of their own; thus, the myeloma cells do not interfere with the production of normal antibody by the fused B cells. Mouse spleen cells are mixed with the myeloma cell line in a ratio of roughly 10:1 in the presence of polyethylene glycol to change membrane permeability and allow cell fusion.

Because cell fusion is random, the cell culture contains a mixture of myeloma–spleen cell fusions, myeloma–myeloma fusions, spleen–spleen cell fusions, single myeloma cells, and single spleen cells. *Selection for only myeloma–spleen cell fusions is accomplished by culturing the cell mixture in hypoxanthine-aminopterin-thymidine (HAT) medium* (see Figure 4-12). Aminopterin is a folic acid analog that blocks the *de novo* biosynthesis of purines and pyrimidines vital for DNA synthesis. Myeloma cells used in hybridoma

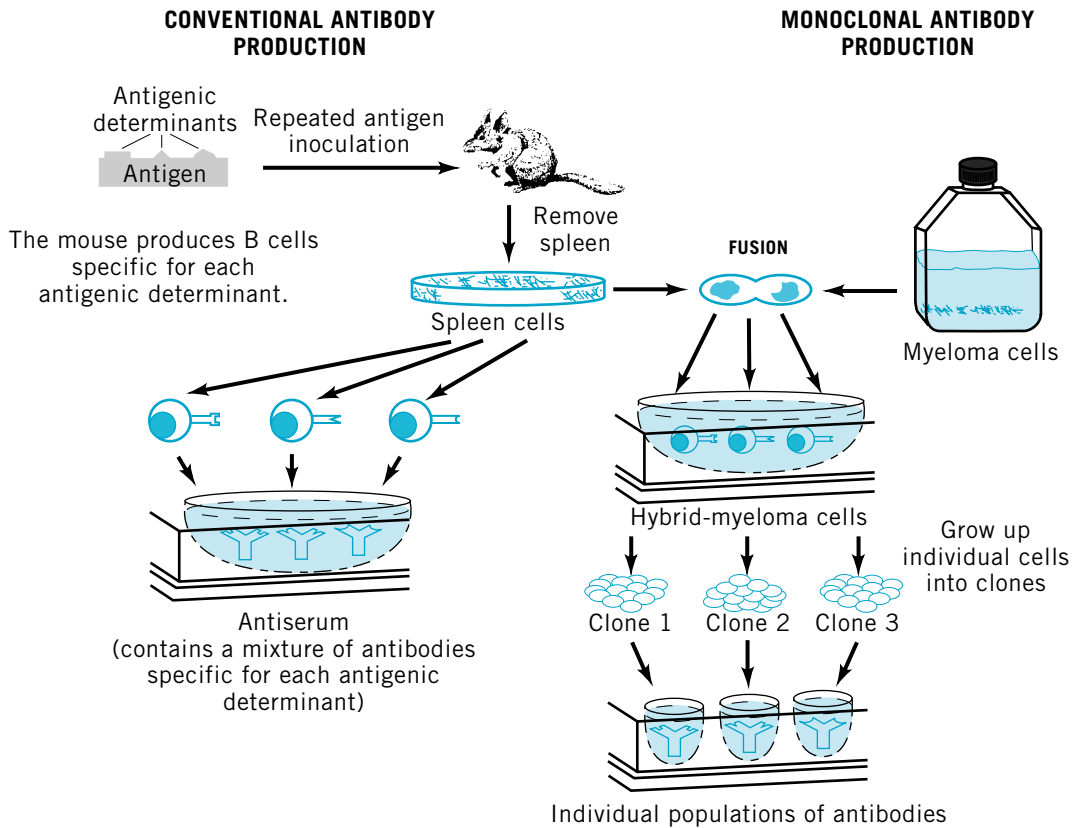


FIGURE 4-11 Conventional antisera compared with monoclonal antibody production. The main difference between the two ways of producing antibodies is the resulting preparations; one is heterogeneous, while the other is homogeneous. B-cell-derived hybridomas can be separated into individual clones and grown indefinitely. One clone gives rise to identical daughter cells, all producing one antibody idiotype (a monoclonal antibody) directed against a specific antigenic determinant. See text for further explanation.

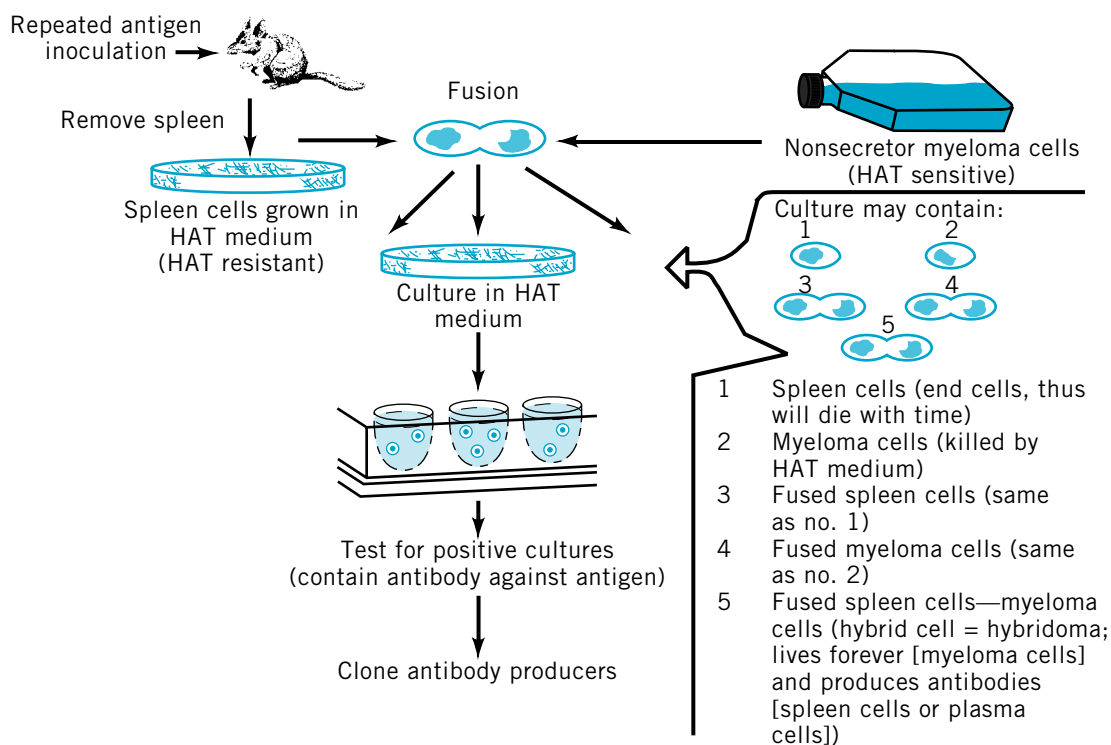
production lack the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT⁻), so they cannot use the exogenous hypoxanthine to synthesize purines by the salvage pathway. Aminopterin also blocks their endogenous synthesis of purines and pyrimidines. In HAT medium, the myeloma–myeloma cells and free myeloma cells die in the first week. Although single spleen cells and spleen–spleen cell fusions express HGPRT and therefore are not selected against by HAT medium, these cells have limited growth in culture and die in 2 weeks. Myeloma cells (HGPRT⁻) that have fused with HGPRT⁺ spleen cells now have the enzyme and can grow in HAT medium. About 500 hybrids are formed per mouse spleen, and 20 to 30 of them produce specific antibody. The correct antibody-producing hybridomas are then identified.

Special Procedures Are Needed to Screen for and Isolate Monoclonal Antibodies

One commonly used method for identifying specific hybridomas is the enzyme-linked immunosorbent as-

say (ELISA) (see Figure 5-9 in Chapter 5). Antigen used to immunize the mice is bound to microculture wells, and supernatants from each of the clones are placed in the antigen-coated wells. After incubation, an enzyme-coupled antibody with specificity for mouse antibodies is added to the wells. After further incubation, the wells are washed to remove unbound antibody, and a substrate for the enzyme is added. If the wells contain antibodies bound to the test antigen, a brightly colored reaction product develops.

Two general methods may be used to isolate individual clones of cells. To clone by limiting dilution, the hybridoma is diluted to a density of 1 to 10 cells per milliliter and dispensed into microculture wells. Seeding cells at such low concentrations implies that statistically each well contains only one clone. Once the clone covers 10 to 50% of the well's surface, the medium can be tested for the presence of antibody. To clone in soft agarose with either fibroblasts or thymocytes added as a feeder layer, hybridoma cells are plated over agarose to immobilize them. Within 10 days, the individual clones are picked out and placed



CELLULAR DNA SYNTHESIS

- 1 *De novo* pathway (endogenous sources)
- 2 Salvage pathway (exogenous sources)

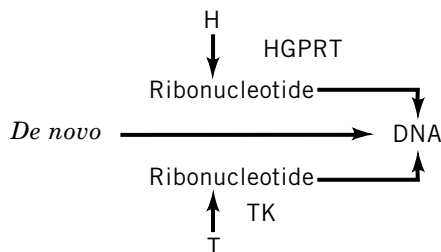


FIGURE 4-12 Standard procedure for the development of monoclonal antibodies.

Hypoxanthine (H) and thymidine (T) are required for the salvage pathway. Aminopterin (A) blocks *de novo* DNA synthesis; thus, myeloma cells deficient in HGPRT enzyme *cannot* grow in HAT medium. However, B-cell-myeloma cell hybrids have the HGPRT enzyme; thus, they can grow because they use the salvage pathway for DNA synthesis. TK, thymidine kinase. See text for further explanation.

in microculture wells with medium. After a week, the clones can be tested for antibody production.

Hybridomas can be grown in large quantities using roller bottles. One liter of spent medium can yield 10 to 100 μg per milliliter of antibody. Alternatively, hybridomas can be grown in the peritoneal cavity of a mouse as an ascites (a tumor that usually grows as a single cell and produces a fluid). Ascitic fluid contains from 1 to 25 mg per milliliter of antibody.

The production of monoclonal antibodies is not as easy as it sounds. From immunization to the establishment of a cloned cell line can take 3 to 6 months of

continuous bench work. The procedure is expensive, time-consuming, labor-intensive, and gives no guarantee of success (**Fast Focus 4**). Immunologists are trying a new method of monoclonal antibody production that uses a cloning system in *Escherichia coli*. If the entire repertoire of an animal can be expressed easily in bacteria, the current methods of producing monoclonal antibodies may become obsolete.

FAST FOCUS 4

Kohler and Milstein could not duplicate their first experiments and only after 6 months of ruined experiments did they discover that the problem was caused by a toxic batch of reagent—the “fun” of REsearch!

Monoclonal Antibodies Have Many Applications

Monoclonal antibodies ushered in a new era in immunologic research and in the application of immunologic assays to basic and clinical questions. The impact of hybridoma technology is obvious when we begin to look at the many situations in which it has been successfully used (Table 4-3).

Monoclonal antibodies are not without problems: (1) the affinity for antigen may be low, (2) individual species of monoclonal antibodies do not readily activate complement or precipitate or agglutinate antigens *in vitro*, and (3) monoclonals are difficult to use *in vivo* in humans because of the difficulty of producing human, as opposed to mouse, hybridomas. To avoid these problems, antibody–gene DNA that has been manipulated *in*

TABLE 4-3 Applications of Hybridoma Technology

1. Generation of routine serologic reagents
2. Monoclonal antibodies against viruses
3. Monoclonal antibodies against parasites
4. Monoclonal antibodies to define mouse and human differentiation and tumor antigens
5. Monoclonal antibodies to the human MHC-encoded antigens
6. In clinical pharmacology, immunosuppressants in transplantation and autoimmune reactions and a targeting vehicle for treatment of cancers
7. In cell biology studies, such as anti-tubulin and anti-actin antibodies
8. In plant physiology studies

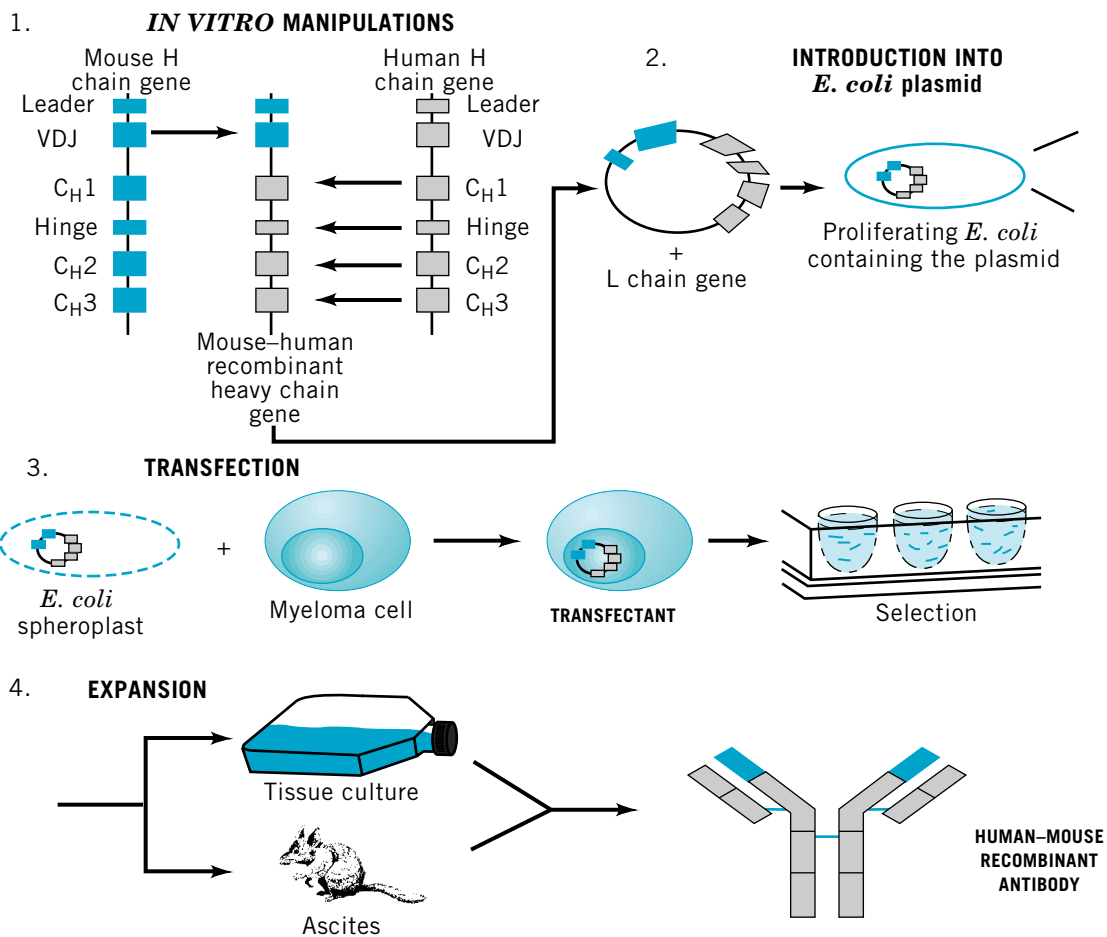


FIGURE 4-13 Transfectomas—the production of a hybrid antibody molecule. The *in vitro* manipulations include ligating the gene containing the sequence of interest (for an antibody, enzyme, toxin, and so on; in this case, combining mouse and human antibody genes into one molecule) into an expression vector such as a eukaryotic cell plasmid. The plasmid is introduced into *E. coli*, the bacteria are grown, and the *E. coli* containing the plasmid with integrated DNA are selected for. These cells are treated with lysozyme to remove the bacterial cell walls, and the resulting spheroplasts are fused (*transfection*) with myeloma cells. After fusion, the stable cells (*transfectants*) are identified and selected. These cells represent the *transfectomas*. Last, the transfectomas are amplified by growing them as ascites or in tissue culture, and the resulting recombinant antibodies are isolated.

in vitro is introduced into myeloma cells. The process is called **transfection**, and the resulting transfected cells are called **transfectomas**. *Transfection is the integration of donor DNA into a cell's chromosomes* (Figure 4-13, see page 74). *If the recipient cell lacks the genetic trait encoded by the donor DNA, the recipient cell acquires that trait.*⁸ Transfection uses several recombinant DNA techniques to produce an entire new family of novel, tailor-made monoclonal antibodies called either **hybrid, chimeric, or recombinant, monoclonal antibodies**. The goal is to *humanize* antibody.

Custom-made antibodies can be molecules with variable regions joined to different isotypic constant regions. These antibodies could enhance the binding of antigen-specific antibodies to protein A (a cell wall component of staphylococci that binds specifically to the Fc portion of IgG molecules) by changing the Fc part, or they could change the antibody's ability to bind complement (keep the same antigen reactivity but change effector function). Custom-made antibodies also can be generated that have unique heavy- and light-chain combinations. This allows one antibody molecule to bind two different antigenic determinants. Furthermore, molecules with Fab antibody sequences can be fused with nonantibody sequences (such as enzyme or toxin sequences). This approach could make chimeric antibodies that are part antibody and part enzyme (which has use in immunoassays) or part toxin (antibody directs toxin to a specific site). The applications of these recombinant antibodies seem limitless.

Why not bypass hybridoma technology and even immunization? You can. Lymphocyte V-region gene repertoires are harvested or assembled *in vitro* and cloned for display of heavy- and light-chain Fab fragments on the surface of bacteriophage, thus the name *phage display antibodies*. Rare phage display antibody is selected by binding to antigen, and soluble Fab fragments are expressed from infected bacteria. Ironically, we are using the bacteria that our immune system is designed to defend against to make antibodies.

MINI SUMMARY

Antigen-specific B cells from an immunized mouse can be fused with immortal B-cell myeloma cells to form hybridomas. Hybridomas have both the specific antibody-producing ability of the B cell and the immortality of the tumor cell. Transfectomas (cells resulting from the integration of donor DNA into the recipient cell's chromosomes) can be generated to produce tailor-made antibodies, called hybrid, chimeric, or recombinant monoclonals.

⁸Transfection is the mammalian counterpart of bacterial transformation.

SUMMARY

Humoral immunity involves a class of **immunoglobulins** called **antibodies**. The electrophoretic mobility of antibodies usually places them in the γ -globulin fraction of serum. Antibodies can directly inactivate antigens and indirectly lead to their destruction through enhanced phagocytosis and complement activation. Antibody classes differ in antigenicity, charge, function, and size. These differences explain antibody functions such as (1) versatility in antigen binding, (2) binding specificity, and (3) biologic activities.

A typical antibody molecule (the prototype class of immunoglobulin is IgG) is made up of four polypeptide chains with a molecular weight of about 150 kD. The four chains are divided into two identical **light chains** and two identical **heavy chains**. An antibody molecule is Y-shaped, with two identical **antigen-binding sites** at the ends of the arms of the Y. The light and heavy chains contribute to the antigen-binding sites. Each antibody molecule can bind to two identical antigenic determinants. Where the arms meet, the stem of the Y is known as the **hinge region**. The hinge region allows **segmental flexibility** of the antibody molecule. The two antigen-binding ends (or amino-terminal ends) of the antibody molecule are called the **Fab fragments** (for fragment antigen binding), whereas the stem (or carboxyl-terminal end) of the Y is considered to be the **Fc fragment** (for fragment crystallizable). The Fc region of the antibody molecule is responsible for its biologic properties, which include activation of the complement system (leading to enhanced phagocytosis), placental transfer, and binding to cell-surface receptors. Myeloma proteins were important in early characterization studies on antibodies.

The amino-terminal end of an antibody is called the **variable, or V, region** and the carboxyl-terminal end is called the **constant, or C, region**. The C region is about the same size as the V region in the light chain and three to four times larger than the V region in the heavy chain. The V regions of light and heavy chains form the antigen-binding sites. Light and heavy chains consist of repeating, similarly folded homology units or **domains**. The light chain has one V-region (V_L) domain and one C-region (C_L) domain, whereas the heavy chain has one V region (V_H) and three or four C-region (C_H) domains. The most variable parts of the V regions are limited to several small **hypervariable regions** or **complementarity-determining regions (CDRs)**. The light- and heavy-chain V regions contain three CDRs. The CDRs come together at the amino-terminal end of the antibody molecule to form the antigen-binding site, which determines *specificity*. The invariant regions of amino acids, between the CDRs,

make up about 85% of the V regions and are designated **framework residues**.

Antigenic determinants that define antibody classes are called **isotypic determinants**, and the classes are referred to as **isotypes**. Isotypic determinants also define **subclasses of antibodies**. The IgG class consists of four subclasses, while the IgA class has two subclasses. Light chains are made up of two classes, κ and λ . Either κ or λ chains can be associated with any class of heavy chain. A $\kappa\lambda$ chain combination never occurs on the same antibody molecule. Only one isotype of the κ chain exists, while there are four isotypes of λ chains. **Allotypic determinants** define antibodies called **allotypes** and are encoded by genes that have several alleles. Allotypes have been characterized for all four subclass chains of IgG, the IgA2 subclass chain, and the κ chain. They are called **Gm**, **Am**, and **Km**, respectively. **Idiotypic determinants** define antibody molecules called **idiotypes**. These determinants are located at the antigen-binding site.

The five different **classes of immunoglobulins** are called **IgG**, **IgA**, **IgM**, **IgD**, and **IgE**, each with a distinctive heavy chain designated γ , α , μ , δ , and ϵ , respectively. IgG is the major antibody in the blood, but it is capable of entering tissue spaces, where it coats antigens, speeding their uptake. IgA concentrates in body fluids to guard the entrances of the body. The extracirculatory dimeric IgA and the circulating IgM are polymerized by a glycopeptide **J chain**. A glycoprotein **secretory component** is added to dimeric IgA by glandular epithelial cells. This antibody molecule is called **secretory IgA**. IgM is the largest antibody; it tends to remain in the blood, where it can efficiently kill bacteria. IgD remains membrane-bound and somehow regulates the cell's activation. IgE is found in trace amounts in the blood and triggers allergies.

When antibody-forming cells are fused with myeloma cells, the resulting clone of cells is a **hybridoma**. Hybridoma cultures are the source of **monoclonal antibodies** of predefined antigen specificity. Use of monoclonal antibodies has revolutionized modern biology. **Transfectomas** (transfected cells) have led to the creation of novel, tailor-made monoclonal antibodies called either **hybrid**, **chimeric**, or **recombinant monoclonal antibodies**.

SELF-EVALUATION

RECALLING KEY TERMS

Allotypes	Km
Allotypic determinants	Antibodies
Am	Antibody subclasses
Gm	Constant (C) region

Fab fragment	Immunoglobulin (or antibody) classes
Fab' fragment	Immunoglobulin domains
F(ab') ₂ fragment	Immunoglobulin gene superfamily
Fc fragment	Immunoglobulins
Framework residues	Isotype
Gm markers	Isotypic determinants
Heavy (H) chain	Joining (J) chain
Hinge region	Km markers
Hybrid, chimeric, or recombinant, monoclonal antibodies	Light (L) chain
Hybridoma	Monoclonal antibodies
Hypervariable regions (or complementarity-determining regions [CDRs])	Multiple myeloma
Hypoxanthine-aminopterin-thymidine (HAT) medium	Secretory IgA
Idiotypes	Segmental flexibility
Idiotypic determinants	Tail pieces
	Transfection
	Transfectomas
	Variable (V) region

MULTIPLE-CHOICE QUESTIONS

(An answer key is not provided, but the page[s] location of the answer is.)

1. A human myeloma protein (IgM κ) is used to immunize a rabbit. The resulting antiserum is then absorbed with a large pool of IgM purified from normal human serum. Following this absorption, the antiserum is found to react only with the particular IgM myeloma protein used for immunization; it is now defined as an anti-idiotypic antiserum. With what specific portion(s) of the IgM myeloma protein would the antiserum react? (a) constant region of the κ chain, (b) variable regions of the μ and κ chains, (c) constant region of the μ chain, (d) J chain, (e) none of these. (See pages 66 and 67)
2. Which of the following statements concerning papain cleavage of IgG is incorrect? (a) the Fc fragment contains part of the heavy chain, (b) the Fab fragment contains both heavy-chain and light-chain determinants, (c) the Fc fragment interacts specifically with certain lymphoid cell receptors, (d) the Fab fragment is divalent, (e) antisera prepared against the Fc fragment are specific for the IgG class of immunoglobulins, (f) none of these. (See pages 60 and 61)
3. The antigen-binding sites of an antibody molecule are located in the (a) C region of light chains, (b) V region of light chains, (c) C region of heavy chains, (d) V region of heavy chains, (e) regions of both light and heavy chains, (f) none of these. (See pages 63, 64, and 67)
4. The class-specific antigenic determinants of immunoglobulins are associated with the (a) light chain, (b) heavy chain, (c) J chain, (d) secretory component, (e) none of these. (See pages 66 and 67)
5. The greater resistance of secretory IgA to proteolytic enzymes is assumed to be a consequence of the (a) binding of the secretory component, (b) predominantly dimeric nature of secretory IgA, (c) antiprotease activity of the heavy chain, (d) presence of the J chain, (e) none of these. (See page 68)
6. IgG is not (a) the major antibody that confers passive immunity on the fetus, (b) divided into five subclasses, (c) made up of

two antigen-binding sites, (d) any of these. (See pages 68 and 70)

7. IgM is (a) not involved in agglutination (clumping) reactions, (b) found as a pentamer on the B lymphocyte's surface, (c) the predominant antibody in the serum, (d) a pentamer in the blood, (e) none of these. (See page 69)
8. IgD (a) is the predominant antibody in colostrum, (b) is present on the surface of all mature B cells, (c) has a molecular weight of 150,000, (d) mediates precipitation reactions, (e) is none of these. (See page 70)
9. IgE (a) increases in individuals with parasitic infections, (b) is the major agglutinating antibody, (c) is *a* and *b*, (d) is none of these. (See page 70)
10. Which of the following represent allotypes? (a) λ light chains, (b) IgA2, (c) Gm, (d) Km, (e) *c* and *d*, (f) none of these. (See pages 66 and 67)
11. Idiotypes are (a) located in the C region of the light chain, (b) located in the C region of the heavy chain, (c) located in and around the antigen-binding region of an antibody molecule, (d) none of these. (See pages 66 and 67)
12. The fusion of what cells leads to a hybridoma cell line that secretes monoclonal antibodies? (a) plasma cells and myeloma cells, (b) monocytes and lymphocytes, (c) lymphocytes and lymphoma cells, (d) plasma cells and activated T cells, (e) none of these. (See pages 71 and 72)
13. Transfectomas are (a) antibodies infected by viruses, (b) hybridomas resulting from fusion induced by viruses, (c) monoclonal antibodies that stop bacterial transfection, (d) cells that produce hybrid antibodies resulting from transfection of bacteria with antibody genes, (e) none of these. (See pages 74 and 75)

SHORT-ANSWER ESSAY QUESTIONS

1. The structure of an antibody molecule is related to its function. Explain.
2. The analysis of antibody molecules by papain and pepsin fragmentation led to similar yet different results. Explain.
3. What is the difference between an immunoglobulin and a myeloma protein? Why were myeloma proteins critical to the early studies on antibody structure?
4. The variability of the amino acid sequence in the antibody's V region was not random but precisely organized. Explain. Differentiate between complementarity-determining regions and framework regions.
5. What are antibody domains?
6. Explain the statements "antibodies can be antigens" or "antibodies are used to characterize antibodies."
7. Briefly discuss immunoglobulin isotypic, allotypic, and idiotypic determinants. Give examples of each.
8. Why can't light chains be used to classify antibodies?
9. List the five classes of antibodies and give a key biologic property of each.
10. Compare conventional antibody production with monoclonal antibody production.
11. In the development of monoclonal antibodies, five different cellular fusion combinations can result. What are they? How can you isolate the appropriate combination?
12. The use of transfectomas to create novel, tailor-made monoclonal antibodies has reduced some of the limitations of monoclonal antibodies. Explain.

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