14 CHAPTER 14 Antibodies: Soluble Antigen Receptors

14.1 KEY POINTS

- There are five classes of immunoglobulins in mammals: IgG, IgM, IgA, IgE, and IgD. All originate as B cell antigen receptors shed into body fluids.
- IgG is the predominant immunoglobulin in serum and is mainly responsible for systemic defense.
- IgM is a very large immunoglobulin produced mainly during a primary immune response.
- IgA is the immunoglobulin produced on body surfaces. It is responsible for the defense of the intestinal and respiratory tracts.
- IgE is found in very small quantities in serum and is responsible for immunity to parasitic worms and for allergies.
- IgD is found on the surface of immature lymphocytes. Its function is unknown.

The properties of the B cell antigen receptors (BCRs) are discussed in Chapter 13. These receptors are, however, not restricted to the B cell surface. Once a B cell response is triggered, the receptors are shed into the surrounding fluid, where they act as antibodies. These antibodies bind to specific antigens and hasten their destruction or elimination. Antibodies are found in many body fluids but are present in highest concentrations and are most easily obtained from blood serum. Antibodies have to defend an animal against a variety of microbes, including bacteria, viruses, and protozoa. They also must act in several different environments—for example, in blood, milk, and body surfaces. It is not surprising, therefore, that several different immunoglobulin classes exist. Each class is optimized for action in a specific environment: for instance, IgA protects body surfaces. Immunoglobulins may also be optimized for activity against a specific group of pathogens. For example, IgE is especially effective against parasitic worms.

14.2 IMMUNOGLOBULINS

Antibody molecules are glycoproteins called immunoglobulins (Ig). The term immunoglobulin is used to describe all soluble BCRs. There are five different classes (or isotypes) of immunoglobulins, which differ in their use of heavy chains. The class found in highest

FIGURE 14-1 The electrophoresis of a protein mixture on a strip of paper or other support. The support bridges two buffer baths, and an electrical potential is applied across them.

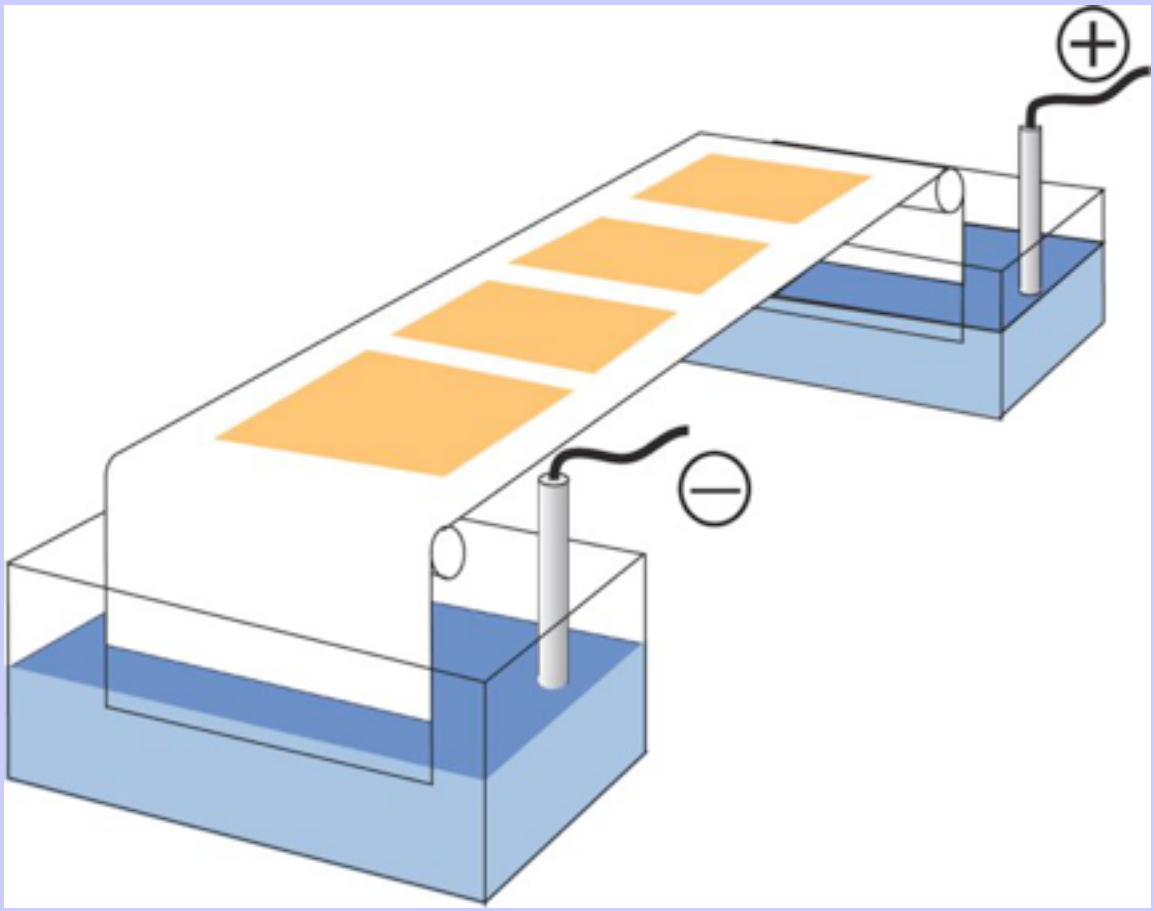
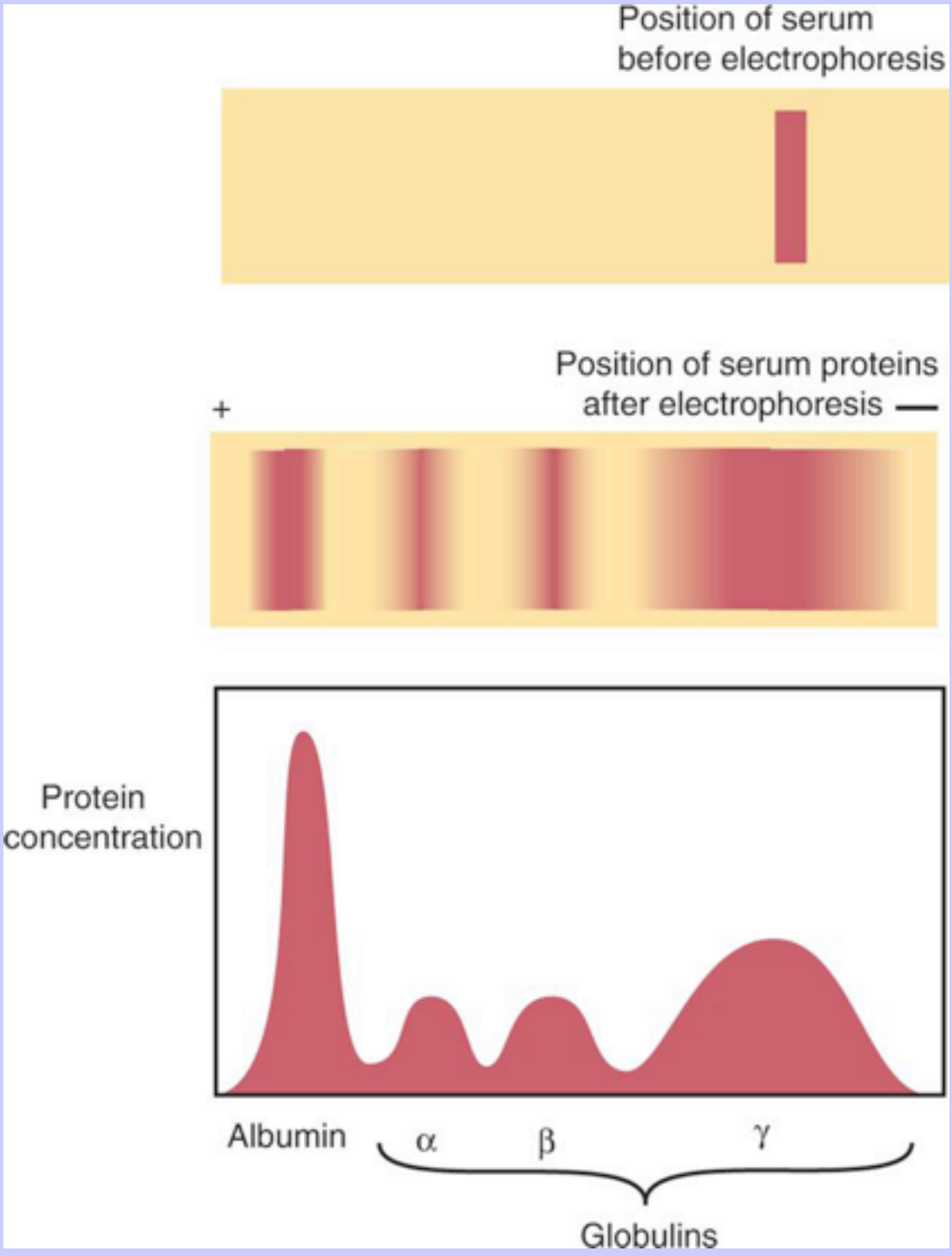


FIGURE 14-2 Schematic diagram showing the results of electrophoresis of whole serum.



concentrations in serum is called immunoglobulin G (IgG). The class with the second-highest serum concentration (in most mammals) is immunoglobulin M (IgM). The third-highest concentration in most mam-mals is immunoglobulin A (IgA). IgA is, however, the predominant immunoglobulin in secretions such as saliva, milk, and intestinal fluid. Immunoglobulin D (IgD) is primarily a BCR and so is rarely encountered in body fluids. Immunoglobulin E (IgE) is found in very low concentrations in serum and mediates allergic reactions. The characteristics of each of these classes are shown in Table 14-1.

Table 14-1 Major Immunoglobulin Classes in the Domestic Mammals

	Immunoglobulin Class				
Property	**IgM**	**IgG**	**IgA**	**IgE**	**IgD**
Molecular weight	900,000	180,000	360,000	200,000	180,000
Subunits	5	1	2	1	1
Heavy chain	μ	γ	α	ε	δ
Largely synthesized in:	Spleen and lymph nodes	Spleen and lymph nodes	Intestinal and respiratory tracts	Intestinal and respiratory tracts	Spleen and lymph nodes

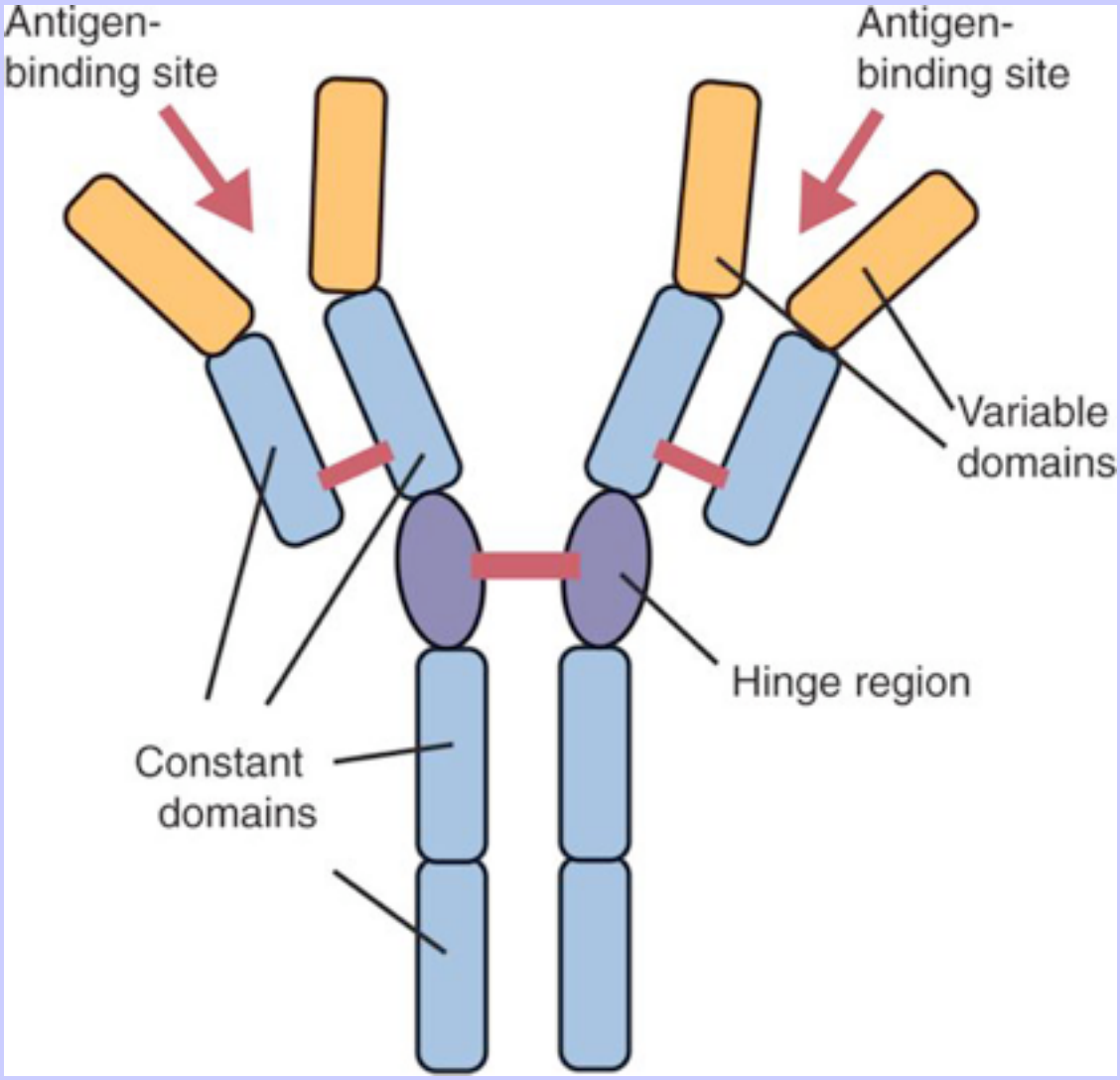
When serum is subjected to electrophoresis, its proteins separate into four major fractions (Figure 14-1). The most negatively charged fraction consists of a single, homogeneous protein called serum albumin. The other three major fractions contain protein mixtures classified as α, β, and γ globulins, according to their electrophoretic mobility (Figure 14-2). Most immunoglobulins are found in the γ globulins, although IgM migrates among the β globulins.

14.3 IMMUNOGLOBULIN CLASSES

14.3.1 Immunoglobulin G

IgG is made and secreted by plasma cells in the spleen, lymph nodes, and bone marrow. It is the immunoglobulin found in highest concentration in the blood (Table 14-2) and for this reason plays the major role in antibody-mediated defense mechanisms. It has a molecular weight of about 180 kDa and a typical BCR structure with two identical light chains and two identical γ heavy chains (Figure 14-3). Its light chains may be of the κ or λ type. Because it is the smallest of the

FIGURE 14-3 The structure of IgG, the prototypical immunoglobulin molecule. Compare this with Chapter 13, Figure 13-6, a typical B cell antigen receptor.



immunoglobulin molecules, IgG can escape from blood vessels more easily than can the others. This is especially important in inflammation, where increased vascular permeability allows IgG to participate in the defense of tissues and body surfaces. IgG binds to specific antigens such as those found on bacterial surfaces. Binding of these antibody molecules to bacterial surfaces can cause clumping (agglutination) and opsonization. IgG antibodies can activate the classical complement pathway only when sufficient molecules have accumulated in a correct configuration on the antigenic surface (see Chapter 5).

Table 14-2 Serum Immunoglobulin Levels in the Domestic Animals and Human

	Immunoglobulin Levels (mg/dl)			
Species	**IgG**	**IgM**	**IgA**	**IgE**
Horse	1000–1500	100–200	60–350	
Cattle*	1700–2700	250–400	10–50	
Sheep	1700–2000	150–250	10–50	
Pig	1700–2900	100–500	50–500	
Dog	1000–2000	70–270	20–150	1–7
Cat†	400–2000	30–150	30–150	
Chicken	300–700	120–250	30–60	
Human	800–1600	50–200	150–400	0.002–0.05

* Cattle show significant seasonal differences in serum immunoglobulin levels.

† Immunoglobulin levels in specific-pathogen-free cats are approximately half those in pet cats.

14.3.2 Immunoglobulin M

IgM is also produced by plasma cells in the spleen, lymph nodes, and bone marrow. It occurs in the second-highest concentration after IgG in most mammalian serum. While on the B cell surface and acting as a BCR, IgM is a 180-kDa immunoglobulin monomer. However, the secreted form of IgM consists of 5 (occasionally 6) 180 kDa subunits linked by disulfide bonds in a circular fashion. Its total molecular weight is 900 kDa (Figure 14-4). A small polypeptide called the J chain (15 kDa) joins two of the units to complete the circle.

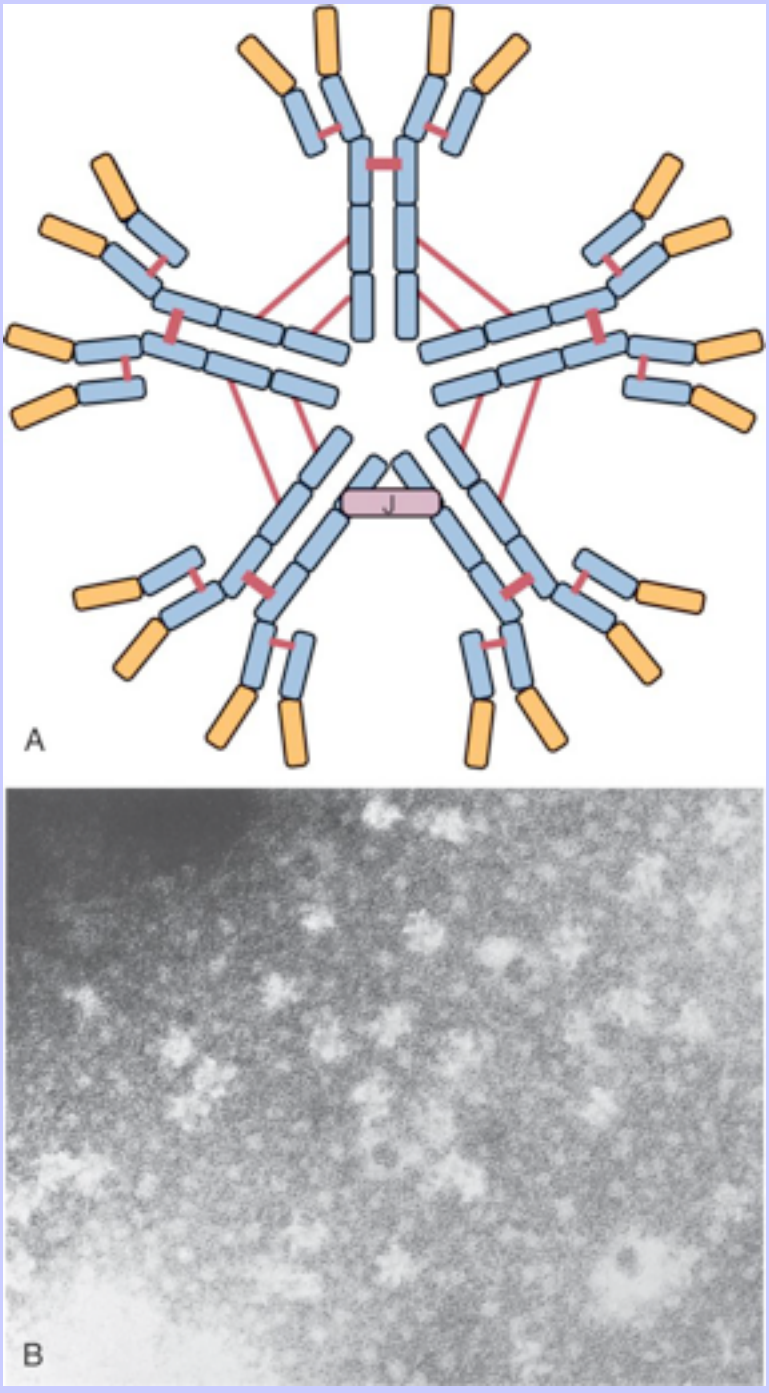
Each IgM monomer is of conventional immunoglobulin structure and so consists of two κ or λ light chains and two μ heavy chains; μ chains differ from γ chains in that they have an additional fourth constant domain (C_H4), as well as an additional 20-amino acid segment on their C terminus but have no hinge region. The complement activation site on IgM is located on the C_H4 domain.

IgM is the major immunoglobulin produced during a primary immune response (Figure 14-5). It is also produced in secondary responses, but this tends to be masked by the predominance of IgG. Although produced in small amounts, IgM is more efficient (on a molar basis) than IgG at complement activation, opsonization, neutralization of viruses, and agglutination. Because they are very large, IgM molecules rarely enter tissue fluids even at sites of acute inflammation.

14.3.3 Immunoglobulin A

IgA is secreted by plasma cells located under body surfaces. Thus it is made in the walls of the intestine,

FIGURE 14-4 The structure of IgM **(A)** and an electron micrograph **(B)** of this immunoglobulin from bovine serum (×240,000). Note that some of the molecules are five-pointed and others are six-pointed stars. (Courtesy Drs. K. Neilsen and B. Stemshorn.)



respiratory tract, urinary system, skin, and mammary gland. Its serum concentration in most mammals is usually lower than that of IgM. IgA monomers have a molecular weight of 150 kDa, but they are normally secreted as dimers. Each IgA monomer consists of two light chains and two a heavy chains containing three constant domains. In dimeric IgA, two molecules are joined by a J chain (Figure 14-6). Higher polymers of IgA are occasionally found in serum.

IgA produced in body surfaces passes through epithelial cells into external secretions. For example, most of the IgA made in the intestinal wall is carried into the intestinal fluid. This IgA is transported through intestinal epithelial cells bound to the polymeric immunoglobulin receptor (pIgR) or secretory component. Secretory component binds IgA dimers to form a complex molecule called secretory IgA (SIgA). It protects the IgA from digestion by intestinal proteases.

Secretory IgA is the major immunoglobulin in the external secretions of nonruminants. As such, it is of critical importance in protecting the intestinal, respiratory, and urogenital tracts, the mammary gland, and the eyes against microbial invasion. IgA does not activate the classical complement pathway, nor can it act as an opsonin. It can, however, agglutinate particulate antigens and neutralize viruses. IgA prevents the adherence of invading microbes to body surfaces. Because of its importance, IgA is examined in more detail in Chapter 19.

14.3.4 Immunoglobulin E

IgE, like IgA, is made by plasma cells located beneath body surfaces. It is a typical Y-shaped, four-chain immunoglobulin with four constant domains in its e heavy chains and a molecular weight of 190 kDa (Figure 14-7). IgE is, however, present in extremely low concentrations in serum. Because of this, it cannot act simply by binding and coating antigens, as the other immunoglobulins do. IgE triggers acute inflammation by acting as a signal-transducing molecule. Thus IgE molecules bind tightly to receptors (FceRI) on mast cells and basophils. When antigen binds to this IgE, it triggers the rapid release of inflammatory molecules from the mast cells. The resulting acute inflammation enhances local defenses and helps eliminate the invader. IgE mediates Type I hypersensitivity reactions and is largely responsible for immunity to parasitic worms. IgE has the shortest half-life of all immunoglobulins (2 to 3 days) and is readily destroyed by mild heat treatment. IgE is described in more detail in Chapter 25.

14.3.5 Immunoglobulin D

IgD has been found in horses, cattle, sheep, pigs, dogs, rodents, and primates but has not yet been detected in rabbits or cats. It has been identified in many different bony fish (catfish, flounder, halibut, carp, salmon, rainbow trout, fugu, zebra fish, and cod) but has not been found in chickens. IgD is a BCR mainly found attached to B cells and very little is secreted into the blood. IgD molecules consist of two δ heavy chains and two light chains but are otherwise structurally diverse. In contrast to the other immunoglobulin classes, IgD is evolutionary labile and shows many variations in structure. For example, mouse IgD lacks a Cd2 domain and thus has only two constant domains in its heavy chains. It has a molecular weight of about

FIGURE 14-5 The relative amounts of each immunoglobulin class produced during the primary and secondary immune responses. Note that IgM predominates in a primary immune response, whereas IgG predominates in a later response.

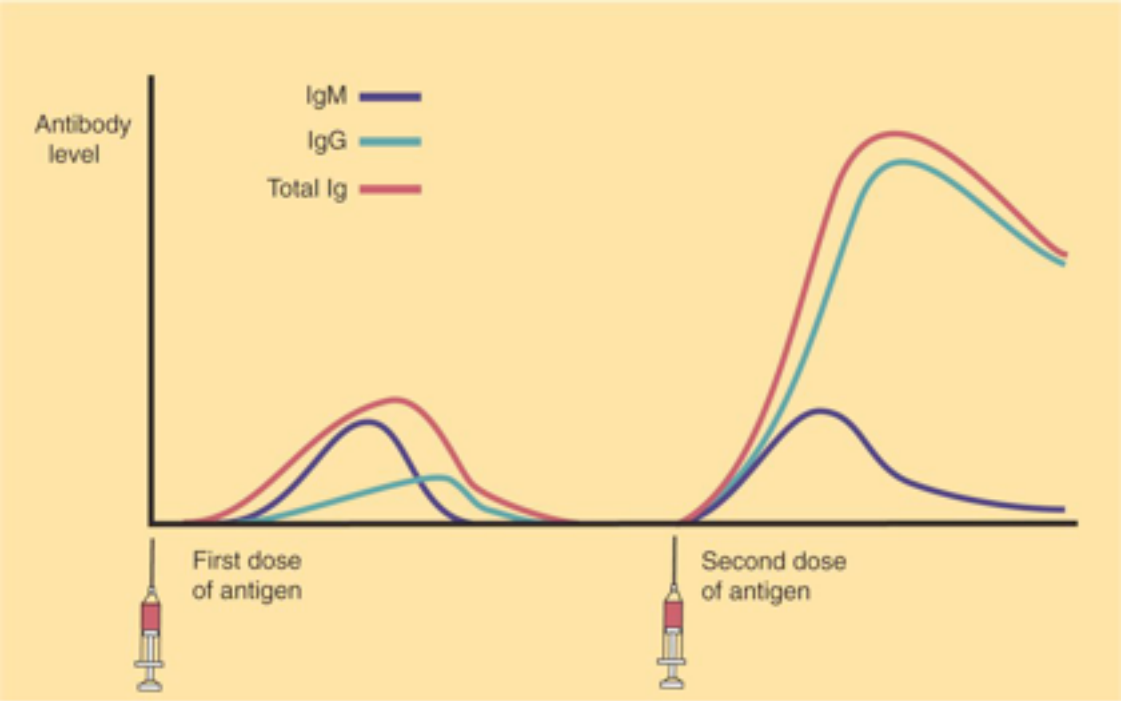


FIGURE 14-6 The structure of IgA and secretory IgA. Secretory component consists of five linked immunoglobulin domains. It is found on the surface of certain epithelial cells, where it acts as a receptor for polymeric immunoglobulins (pIgR). It can also bind to IgM.

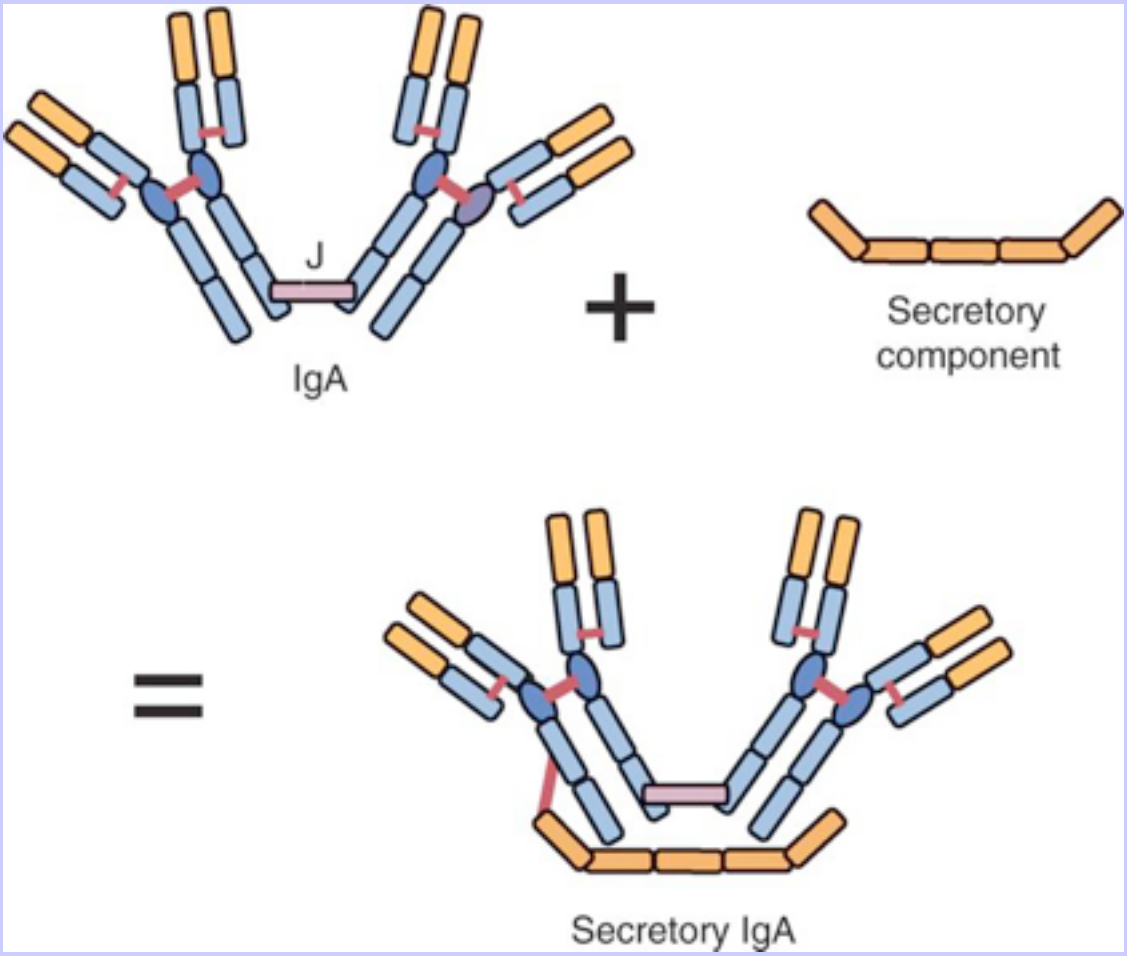
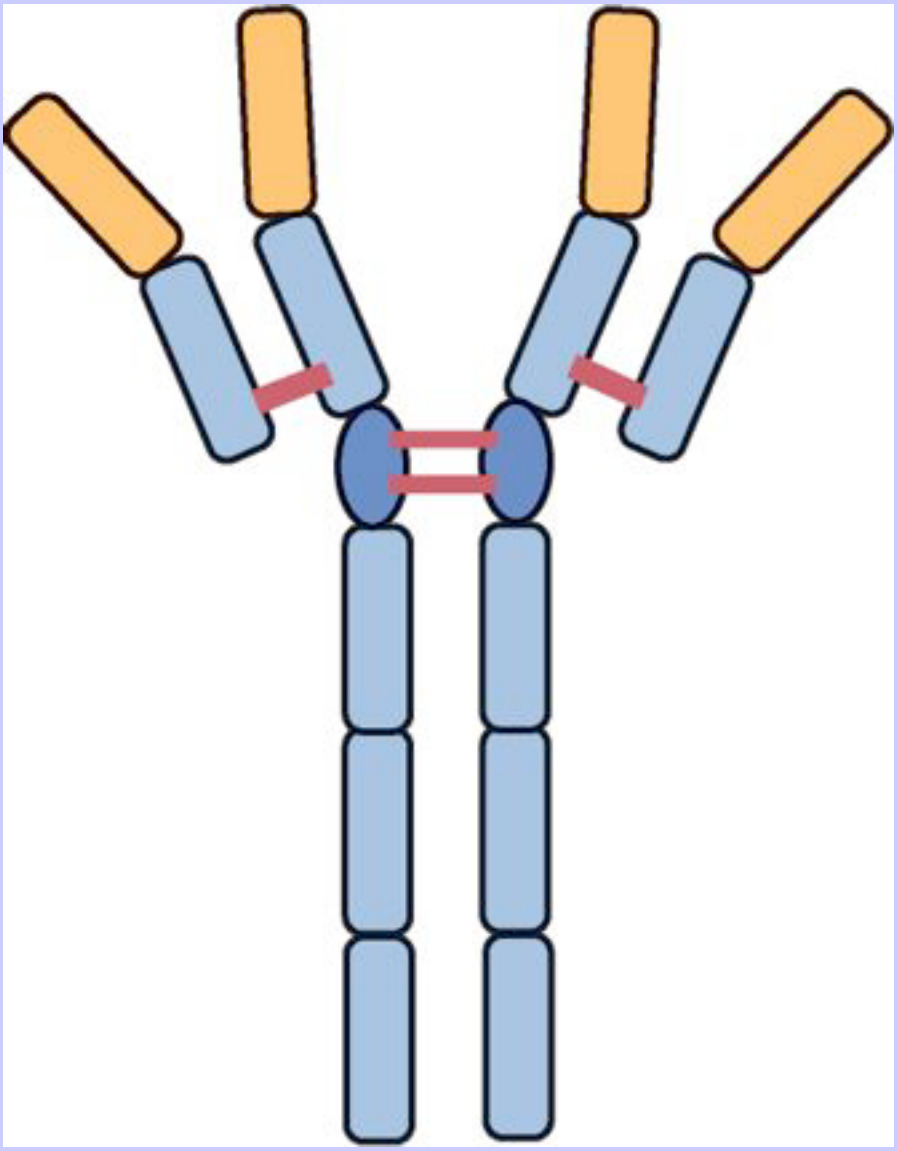


FIGURE 14-7 The structure of IgE. Note the presence of four constant domains in addition to a hinge in the heavy chain.



170 kDa (Figure 14-8). Horse, cow, sheep, dog, monkey, and human IgD, in contrast, have three heavy chain constant domains and a very long hinge domain coded for by two exons (Figure 14-9). Pig IgD has a short hinge coded for by a single exon. In cattle, sheep, and pigs, but not horses or dogs, the Cd1 domain is almost identical to the Cm1 domain of IgM, whereas the other constant domains are distinctly different. In mice, the two constant region domains (Cd1 and Cd3) are separated by a very long exposed hinge region. Because of this long hinge region and the fact that it has no interchain disulfide bonds, mouse IgD is unusually susceptible to destruction by proteases and cannot be detected in mouse serum although it may be detected in mouse plasma. Like IgE, IgD is destroyed by mild heat treatment.

14.4 THREE-DIMENSIONAL STRUCTURE OF IMMUNOGLOBULINS

Immunoglobulin peptide chains fold in a very complex manner so that an IgG molecule consists of three globular regions (two Fab regions and one Fc region) linked

174

175

FIGURE 14-8 The structure of IgD in mice and other mammals. Note the long exposed hinge region in mouse IgD that makes this molecule very unstable.

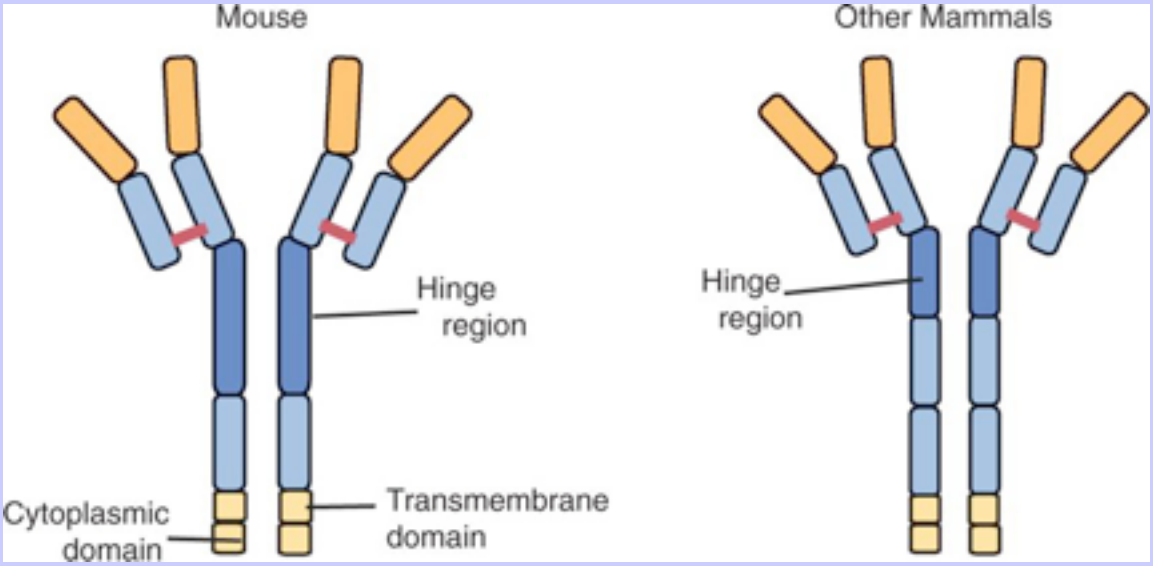
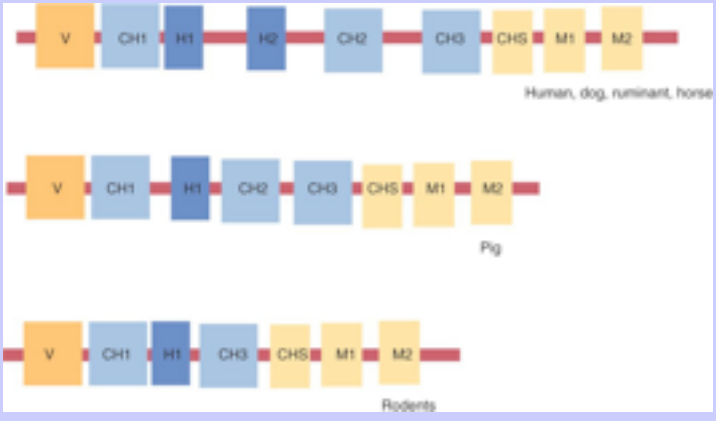


FIGURE 14-9 The gene structure of IgD differs greatly between mammals. This diagram shows the exon structure of IgD heavy chains in different species. No other immunoglobulin class shows such variation and its significance is unknown.



by a flexible hinge (Figure 14-10). Each of these globular regions is made up of paired domains. Thus the Fab regions each consist of two interacting domains (V_H-V_L and C_H1-C_L), while the Fc region contains either two or three paired domains, depending on the immunoglobulin class (i.e., C_H2-C_H2, C_H3-C_H3, and in IgE or IgM, C_H4-C_H4). The peptide chains within each domain are closely intertwined. In the Fab globular regions a groove is located between the two variable domains, V_H and V_L. The amino acids of the complementarity-determining regions (CDRs) line this groove; as a result, the surface of the groove has a highly variable shape. This groove forms the antigen-binding site. The CDRs from both light and heavy chains contribute to the binding of an antigen, although the heavy chain usually contributes most to the process. Because immunoglobulins are bilaterally identical, the CDRs on each of the Fab regions are also identical. Thus the molecule has two identical antigen-binding sites and binds two identical antigens.

The presence of a hinge region in the middle of their heavy chains gives immunoglobulins such as IgG great flexibility. Since the two antigen-binding sites on each Fab region are identical, immunoglobulins are able to cross-link two antigens at the same time. Thus bacteria may be clumped together by antibody molecules in a process called agglutination. If sufficient soluble protein molecules or viruses are cross-linked by antibody, they may precipitate out of solution.

175

176

14.5 IMMUNOGLOBULIN VARIANTS

14.5.1 Subclasses

All immunoglobulin molecules are made of two heavy and two light chains. Several different heavy chains are employed in making these molecules. Thus when γ chains are used, the resulting immunoglobulin is IgG. IgM contains μ chains, IgA contains a chains, and so on. However, closer examination shows that even these immunoglobulin classes consist of molecules using a mixture of structurally different heavy chains known as subclasses.

Immunoglobulin subclasses have arisen as a result of gene duplication. Thus during the course of evolution, heavy chain *(IGH)* genes have been duplicated and the new gene then gradually changed through mutation. The amino acid sequences coded by these new genes may differ from the original in only minor respects. For example, bovine IgG is a mixture of three subclasses—IgG1, IgG2, and IgG3—coded for by the genes *IGHG1, IGHG2,* and *IGHG3,* respectively. They differ in amino acid sequence and in physical properties such as electrophoretic mobility. These immunoglobulin subclasses may also have different biological activities: for example, bovine IgG2 agglutinates antigenic particles, whereas IgG1 does not. All animals of a species will possess all these subclasses.

The number and properties of immunoglobulin subclasses vary among species. For example, most mammals have only 1 or 2 IgA subclasses, but rabbits have as many as 13. These variations among species are probably not of major biological significance; they simply reflect the number of immunoglobulin gene duplications a species has undergone.

14.5.2 Allotypes

In addition to subclass differences, individual animals show inherited variations in immunoglobulin amino acid sequences. Thus the immunoglobulins of one individual may differ from those of another individual of the same species (Figure 14-11). These inherited sequence variations in heavy chain genes are called allotypes.

14.5.3 Idiotypes

The third group of structural variants found in immunoglobulins results from the variations in the amino acid sequences within the variable domains on light

FIGURE 14-10 A computer-generated molecular model of IgG. It is instructive to compare this with the diagrams of IgG structure seen earlier in this chapter. (Courtesy Dr. S. Linthicum.)

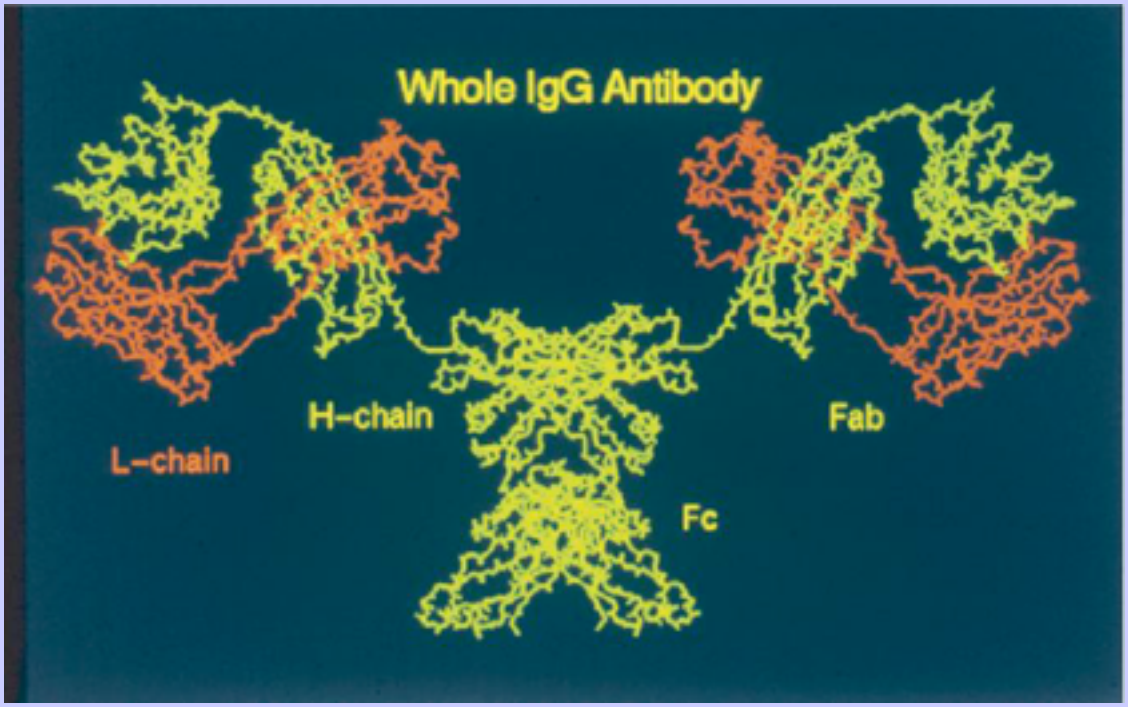
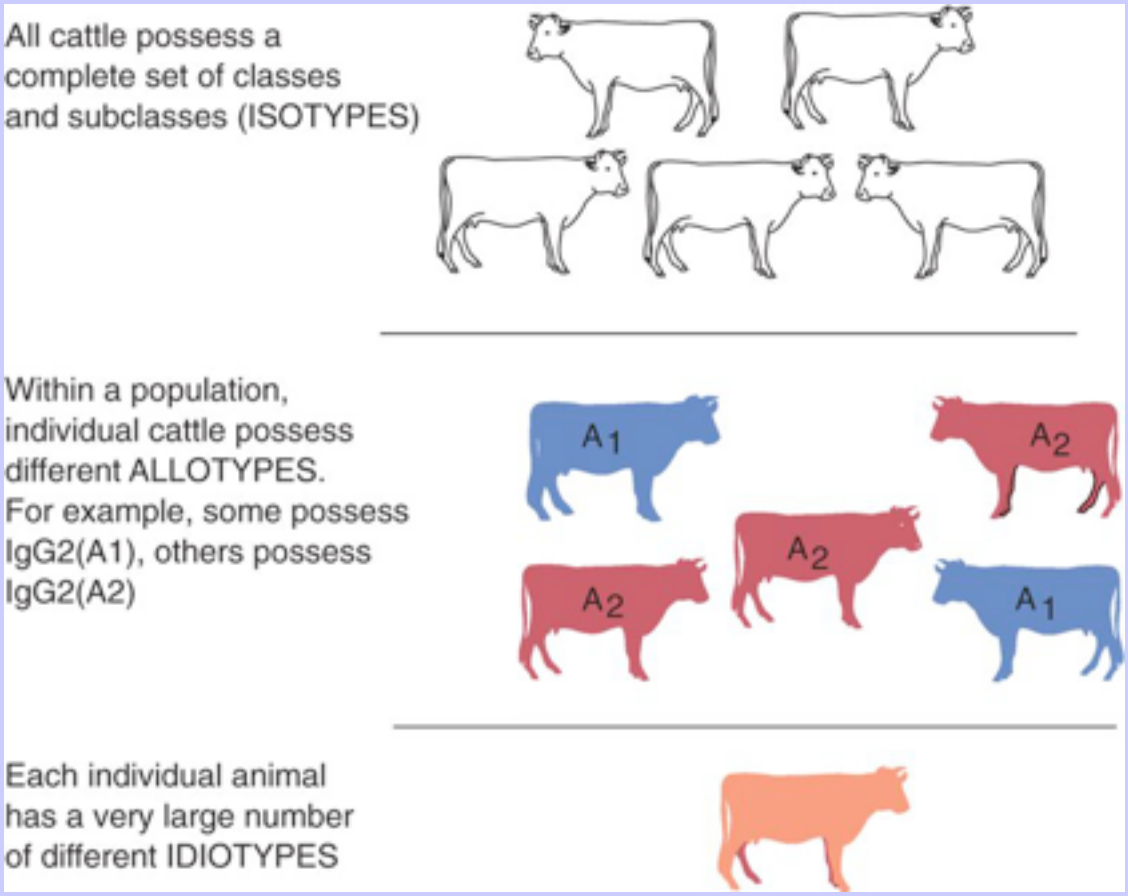


FIGURE 14-11 A schematic diagram showing the differences among the inheritance of the major immunoglobulin variants.



176

and heavy chains. These variants are called idiotopes. The collection of idiotopes on an immunoglobulin is called its idiotype. Some idiotopes may be located within the antigen-binding site. Others are located on nonantigen binding areas of the V domain. 177

14.6 PRODUCTION OF IMMUNOGLOBULIN HEAVY CHAINS

Two different genes code for each immunoglobulin heavy chain. One gene codes for the variable domain (and thus the antigen-binding site), whereas a separate gene codes for the constant domains. The way in which genes can code for the variable domains is discussed in Chapter 15. The genes that code for the constant region of immunoglobulin heavy chain (*IGH* genes) each consist of several exons (expressed sequences). Each exon codes for a constant domain, and one codes for the hinge region (Figure 14-12). A complete IgM constant region gene (*IGHM*) therefore consists of five exons, whereas an IgA constant region gene (*IGHA*) contains four exons. All the heavy chain constant region genes are located together on one chromosome. They are generally arranged in the order 5′-*IGHM-IGHD-IGHG-IGHE-IGHA*-3′. Thus all the genes for μ chains are followed by the genes for δ chains, which are in turn followed by the γ chain genes and so on.

During their life span, B cells undergo two different DNA recombination events. The first, called V(D)J recombination, creates the antigen binding site of the B cells as they develop within the bone marrow in the absence of antigens. Later in life, when antigens activate the B cells, a second phase of DNA recombination occurs. This second phase changes the class of antibody produced by a B cell. This class switch recombination does not affect the antigen-binding specificity of a cell but results in the production of a different heavy chain constant region.

14.6.1 Class Switch Recombination

During the course of an antibody response, immunoglobulin classes change, although their antigen-binding ability does not. This “class switch” can be explained by the way in which heavy chain genes are constructed and used.

During an antibody response, the immunoglobulin classes are synthesized in a standard sequence. Thus a B cell first uses the *IGHM* genes to make IgM BCRs. The remaining genes located 3′ to *IGHM* are ignored. In species that make IgD, the B cell also transcribes the *IGHD* genes and then expresses both IgM and IgD. Eventually, however, as the immune response progresses, a responding B cell switches to using *IGHG, IGHA,* or *IGHE* genes and becomes committed to synthesizing BCRs and immunoglobulins of one of the other major classes—namely, IgG, IgA, or IgE. The unwanted, unused IGH genes are excised as a DNA circle and lost from the cell while the required IGH gene is spliced directly to the IGHV genes.

For example, if IgM is to be synthesized, the IGHV genes are spliced directly to the IGHM genes (Figure 14-13). On the other hand, if IgA is to be synthesized, the genes coding for Cm to Ce inclusive are deleted and the IGHV genes are then spliced directly to the IGHA genes. There are several ways by which these

FIGURE 14-12 A peptide chain such as an immunoglobulin heavy chain is coded for by a series of exons separated by intervening sequences or introns. Usually each exon codes for a single domain.

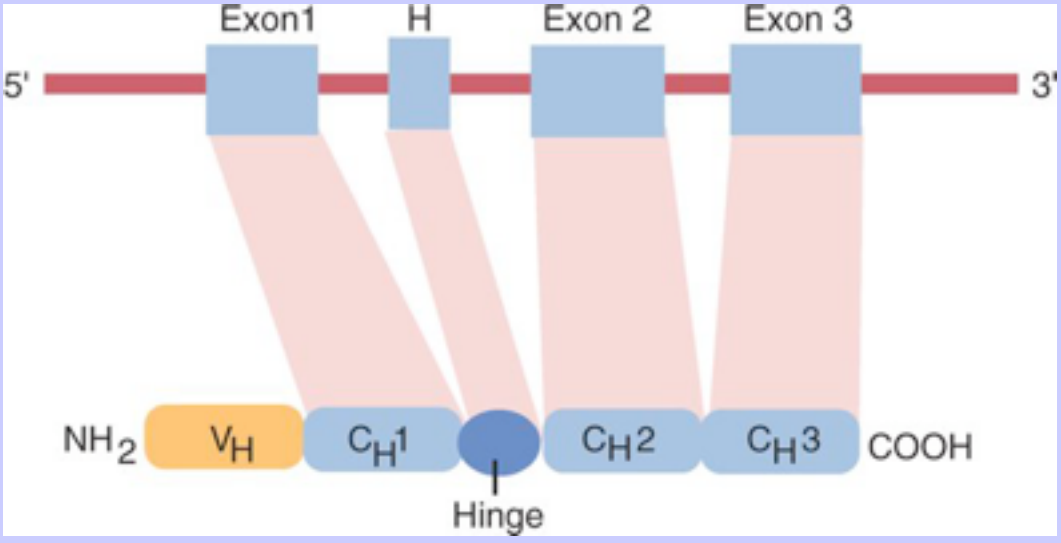
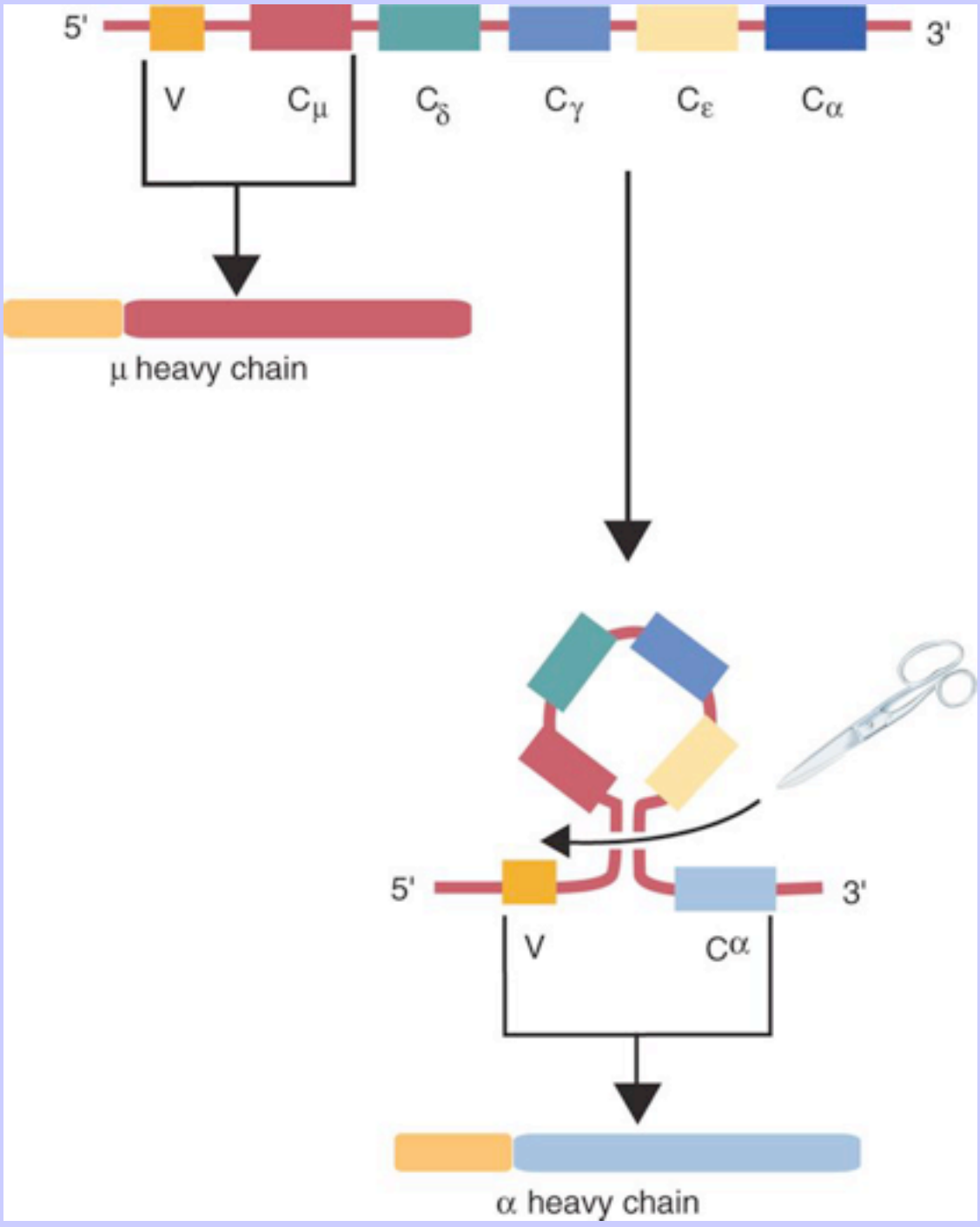


FIGURE 14-13 The mechanism of class switching. In this example a switch is made from IgM production to IgA production.



177

intervening genes can be excised. The simplest is called looping out-deletion. In this case the V region and C gene segments come together by looping out and then excising the intervening DNA using an enzyme called a

178

recombinase. Two signals are needed to initiate class switching in a B cell. First, the B cell must receive an activation signal. This comes from cross-linking between CD40 on the B cell and CD154 on a helper T cell. Second, the specific class switch must be determined. This choice is regulated by cytokines, especially by interleukin-4, transforming growth factor-β, and interferon-g. Signals from the CD40 and antigen activate the recombinase in the B cell while the cytokines, by activating specific promoter regions, target the recombinase to a specific immunoglobulin gene.

14.6.2 BCRs and Soluble Immunoglobulins

Immunoglobulins can exist either as BCRs or as secreted antibodies. The heavy chain of a BCR contains a hydrophobic transmembrane C-terminal domain that attaches it to a B cell. This domain is absent from the secreted antibody. The switch between the two forms depends on the differential splicing of exons. For example, in the *IGHM* gene, there are two short exons, CμS and CμM, located 3′ to Cμ4 (Figure 14-14). CμS codes for the C-terminal domain of the secreted form, whereas CμM codes for the hydrophobic domain of the cell-bound form. When IgM is made, all the Cm exons are first transcribed to mRNA. To produce cell-bound IgM, the mRNA is cleaved so that the CμS exon is deleted and the Cμ4 exon is spliced directly to the CμM exon. To produce secreted IgM, the exon coding for the CμM domain is deleted and translation is stopped after Cμ4 and CμS are read.

14.7 IMMUNOGLOBULINS OF DOMESTIC MAMMALS

All mammals possess genes for and express four or five major immunoglobulin classes (IgG, IgM, IgA, IgE, and IgD), although these may not have been formally identified in all species (Table 14-3). The basic characteristics of each of these classes are as described previously. However, during the course of evolution, as pointed out above, the immunoglobulin heavy chain (IGH) genes have duplicated, sometimes several times. These duplicated genes can then mutate so that mammals may produce several different subclasses of a specific immunoglobulin. If a duplicated gene mutates in such a way that it is no longer functional, it becomes a pseudogene. The number of duplications and hence the number of immunoglobulin subclasses and pseudogenes varies greatly among species. In looking at these species differences, the reader might gain additional insight by examining the phylogeny of domestic animal species (see Figures 37-13 and 37-14).

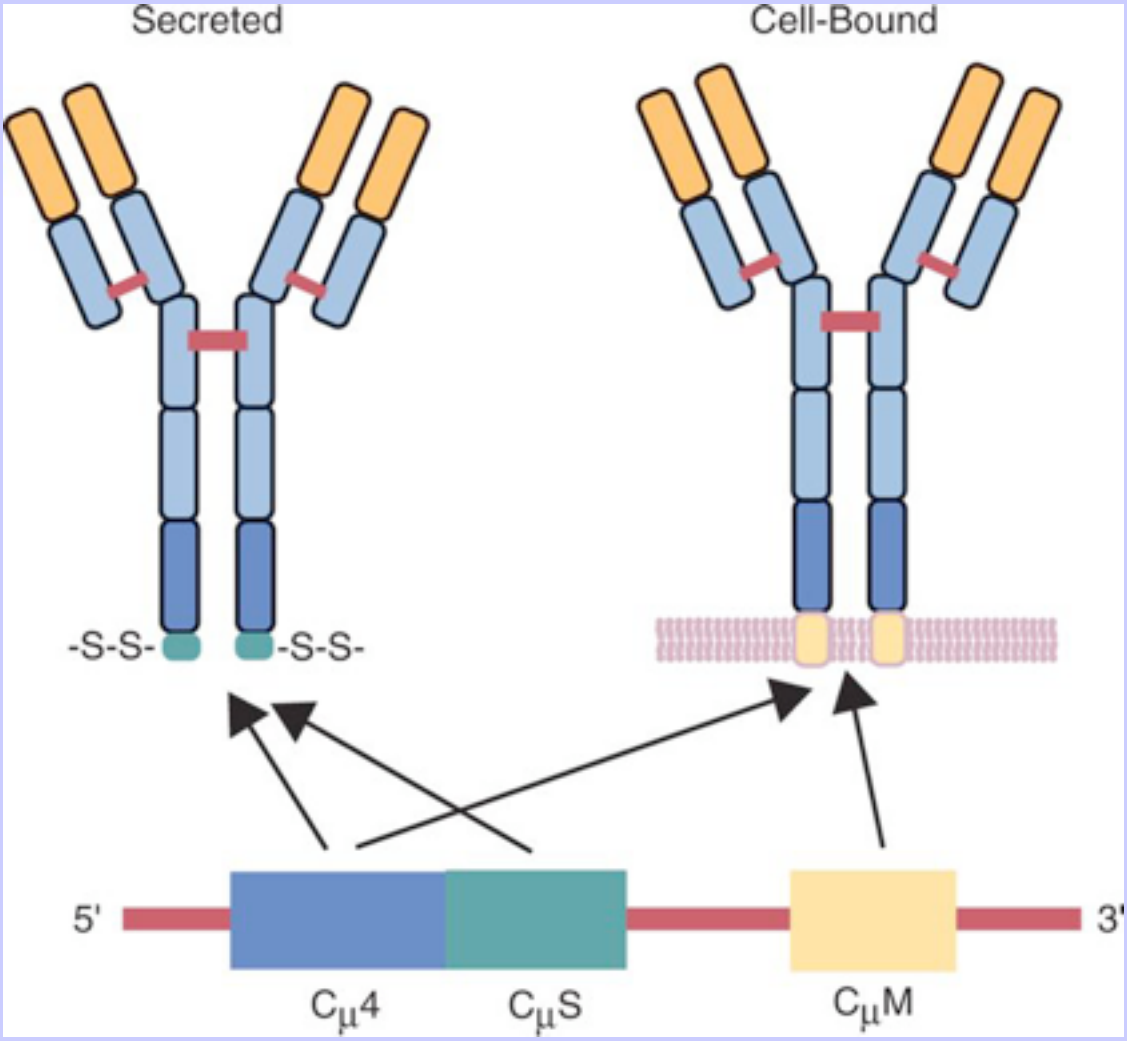
14.7.1 Horses

The horse has seven IGHG genes, and all are expressed. Thus there are seven IgG subclasses: IgG1 (IgGa), IgG2 (IgGc), IgG3 (IgG[T]), IgG4 (IgGb), IgG5, IgG6 (IgG[B]), and IgG7. (The previously used designation for IgG3—IgG[T]—was originally derived from the observation that this subclass predominates in the serum of horses used for tetanus immune globulin production.) IgG3 does not activate guinea pig complement and reacts in a precipitation reaction by a rather characteristic flocculation. The order of the Ig heavy chain genes in the horse is now: 5′-M-D-G1-G2-G3-G7-G4-G6-G5-E-A-3′. The gene, coding for IgG7, is closely related to IGHG4 and likely emerged from a recent duplication of the IGHG4 gene. The horse heavy chain gene locus is located on chromosome 24qtr. This corresponds to human chromosome 14, where the human IGH locus is located. Horses also possess and express IgM, IgD, IgA, and IgE. The horse IGHD gene is located downstream from IGHM. It appears to be expressed at least at the mRNA level. Horses have two IgG4 allotypes ($IgG4^{a}$ and $IgG4^{b}$) and four IgE allotypes ($IgE^{1–4}$).

14.7.2 Cattle

Cattle have three *IGHG* genes and thus three subclasses: IgG1, IgG2, and IgG3. IgG1 constitutes about

FIGURE 14-14 Immunoglobulins serving as B cell antigen receptors have a hydrophobic transmembrane C-terminus. In contrast, the secreted form lacks this sequence. The difference between the two forms is determined by RNA splicing following transcription.



178

50% of the serum IgG and is remarkable for being the predominant immunoglobulin in cows' milk rather than IgA. IgG2 levels are highly heritable; thus concentrations vary greatly among cattle. Cattle possess a unique Fc receptor on their macrophages and neutrophils that is structurally unlike any other Fc receptor and binds only IgG2. Since bovine IgG2 has a very small hinge region, this receptor might represent a special adaptation to the

179

structure of this immunoglobulin. Two heavy chain allotypes (a and b) have been identified in all three classes. Allotype B1 is found on light chains of some cattle but is relatively uncommon. IgA, IgM, and IgE also occur in cattle. Cattle have functional *IGHD* genes, and IgD may be expressed on the B cell surface. Cattle are also unique in that they have two *IGHM* genes, although one, the *IGHML*-gene, is a pseudogene located on chromosome 9. The functional *IGHM* gene is located on chromosome 21 together with the other heavy chain genes.

Table 14-3 Immunoglobulin Classes and Subclasses in Selected Mammals

	Immunoglobulin Classes				
Species	**IgG**	**IgA**	**IgM**	**IgE**	**IgD**
Horse	G1, G2, G3, G4, G5, G6, G7	A	M	E	D
Cattle	G1, G2, G3	A	M	E	D
Sheep	G1, G2, G3	A1, A2	M	E	D
Pig	G1, G2a, G2b, G3, G4	A	M	E	D
Dog	G1, G2, G3, G4	A	M	E1, E2	D
Cat	G1, G2, G3, (G4?)	A	M	(E1, E2?)	?
Mouse	G1, G2a, G2b, G3	A1, A2	M	E	D
Chimpanzee	G1, G2, G3,	A	M	E	D
Human	G1, G2, G3, G4	A1, A2	M1, M2	E	D

14.7.2.1

Box 14-1 The Curious Case of the Camel

Members of the camel family from both the old and new worlds (camels and llamas) have three IgG subclasses: IgG1, IgG2, and IgG3. IgG1 has a conventional four-chain structure and therefore has a molecular weight of 170 kDa. In contrast, IgG2 and IgG3, which together account for 75% of camel immunoglobulins, are 100-kDa heavy chain dimers that have no light chains. In addition, camel IgG2 heavy chains lack a CH1 domain but compensate for this by having a very long hinge region. Despite lacking light chains, these molecules can still bind to many antigens. It has been noted that these antibodies appear to bind to the substrate pockets of enzymes. Studies have now shown that the antigen-binding site on these heavy chains (the paratope) is very convex. This enables it to fit snugly into the concave active site on an enzyme. Thus these single chain antibodies may have a structural advantage over conventional immunoglobulins in neutralizing enzyme activity.

14.7.3

Sheep

The immunoglobulin subclasses of sheep are similar to those of cattle, with three *IGHG* genes coding for IgG1, IgG2, and IgG3. Some sheep have an IgG1a allotype. An *IGHD* gene has been detected in sheep. Three IgA heavy chain allotypes have been identified.

14.7.4 Pigs

Pigs have at least five IgG subclasses—named IgG1, IgG2a, IgG2b, IgG3, and IgG4. Whether all deserve to be called subclasses is unclear. For example, IgG2a and IgG2b differ by only three amino acids. However, DNA analysis has indicated that pigs may have 8 to 12 distinct *IGHG* genes. Presumably the unused genes are pseudogenes. IgG is the predominant serum immunoglobulin accounting for about 85% of the total. IgM accounts for about 12%, and dimeric IgA for about 3% of serum immunoglobulins. Pigs have a single *IGHA* gene that occurs in two codominant allelic variants. One form, *IGHAa,* has a normal hinge region with six amino acids. The other allele, *IGHAb,* has a deletion mutation so that its hinge contains only two amino acids. The biological consequences of this are unclear. The first heavy chain constant domain of pig IgD may be coded by either a CH1 δ gene or by a CH1 μ gene. Thus pig IgD heavy chains can be coded by "VDJ-CH1μ-CH2δ-CH3δ," or by "VDJ-CH1δ-CH2δ-CH3δ." This pattern has not been reported in other mammals. These two genes do however show 98.7% similarity, so the biological consequences are probably not great. Swine IgE has also been identified. Four IgG allotypes and one IgM allotype have been reported (Box 14-1).

179

180

14.7.5 Dogs and Cats

Dogs have four *IGHG* genes and hence four IgG subclasses, named IgG1, IgG2, IgG3, and IgG4 in order of abundance. (These were previously called IgG-A, -B, -C, and -D). In addition, dogs have IgA, IgM, IgD, and IgE. Preliminary evidence also suggests that they may have two IgE subclasses, IgE1 and IgE2. Four allelic variants have been identified in the dog IGHA gene. All are restricted to the hinge region.

Cats have at least three, and possibly four, *IGHG* genes (IgG1, IgG2, IgG3, and IgG4), one IgM subclass, and possibly two IgA subclasses (IgA1 and IgA2), as well as two possible IgE subclasses. An IgM allotype has been described in the dog.

14.7.6 Primates

Humans have four *IGHG* genes coding for IgG1 to IgG4. Chimpanzees and rhesus macaques possess three *IGHG* genes coding for IgG1, IgG2, and IgG3. The chimpanzee IgG2 molecule contains epitopes also found on both human IgG2 and IgG4, suggesting that the *IGHG2* and *IGHG4* genes split after humans separated from chimpanzees. Baboons *(Papio cynocephalus)* have four *IGHG* genes, but they differ significantly from human IgG in their hinge region. Rhesus macaques may have two IgM subclasses. All the great apes, with the exception of the orangutan, have two IgA subclasses.

14.7.7 Other Mammals

Rats and mice have four or five functional *IGHG* genes. In contrast, rabbits have only one *IGHG* gene despite having 13 *IGHA* genes, at least 12 of which are functional. They appear to lack IgD. The expression of these IgA subclasses varies among different tissues

14.8 SOURCES OF ADDITIONAL INFORMATION

CI Baldwin, DA Denham: Isolation and characterization of three subpopulations of IgG in the common cat (Felis catus). *Immunology*. **81**, 1994, 155–160.

DR Burton: Antibody: the flexible adaptor molecule. *Trends Biochem Sci*. **15**, 1990, 64–69.

JE Butler, WR Brown: The immunoglobulins and immunoglobulin genes of swine. *Vet Immunol Immunopathol*. **43**, 1994, 5–12.

KE Conrath, U Wernery, S Muyldermans, VK Nguyen: Emergence and evolution of functional heavy-chain antibodies in Camelidae. *Dev Comp Immunol*. **27**, 2003, 87–103.

E De Genst, K Silence, K Decanniere, et al.: Molecular basis for the preferential cleft recognition by dromedary heavy-chain antibodies. *Proc Natl Acad Sci*. **103**, 2006, 4586–4591.

AP Foster, WPH Duffus, SE Shaw, TJ Gruffydd-Jones: Studies on the isolation and characterization of a reaginic antibody in a cat. *Res Vet Sci*. **58**, 1995, 70–74.

CK Grant: Purification and characterization of feline IgM and IgA isotypes and three subclasses of IgG. In BJ Willett, O Jarrett (Eds.): *Feline immunology and immuno-deficiency*. 1995, Oxford University Press, Oxford, England.

C Hamers-Casterman, T Atarhouch, S Muyldermans, et al.: Naturally occurring antibodies devoid of light chains. *Nature*. **363**, 1993, 446–448.

I Kacskovics, J Sun, JE Butler: Five putative subclasses of swine IgG identified from the cDNA sequence of a single animal. *J Immunol*. **153**, 1994, 3565–3573.

J Naessens, J Newson, DJL Williams, V Lutje: Identification of isotypes and allotypes of bovine immunoglobulin M with monoclonal antibodies. *Immunology*. **63**, 1988, 569–574.

G Overesch, B Wagner, A Radbruch, W Leibold: Organization of the equine immunoglobulin constant heavy chain genes. II. Equine Cg genes. *Vet Immunol Immunopathol*. **66**, 1998, 273–287.

J Purkerson, P Isakson: A two-signal model for regulation of immunoglobulin isotype switching. *FASEB J*. **6**, 1992, 3245–3252.

JM Roe, D Patel, KL Morgan: Isolation of porcine IgE and preparation of polyclonal antisera. *Vet Immunol Immunopathol*. **37**, 1993, 83–97.

L Tang, C Sampson, MJ Dreitz, C McCall: Cloning and characterization of cDNAs encoding four different canine immunoglobulin gamma chains. *Vet Immunol Immunopathol*. **80**, 2001, 259–270.

B Wagner, DC Miller, TL Lear, DF Antczak: The complete map of the Ig heavy chain constant gene region reveals evidence for seven IgG isotypes and for IgD in the horse. *J Immunol*. **173**, 2004, 3230–3242.

M Yang, AB Becker, FE Simons, Z Peng: Identification of a dog IgD-like molecule by a monoclonal antibody. *Vet Immunol Immunopathol*. **47**, 1995, 215–224.

G Zhang, JR Young, CA Tregaskes, et al.: Identification of a novel class of mammalian Fcg receptor. *J Immunol*. **155**, 1995, 1534–1541.

Y Zhao, I Kacskovics, Q Pan, et al.: Artiodactyl IgD: the missing link. *J Immunol*. **169**, 2002, 4408–4416.

Y Zhao, Q Pan-Hammarström, I Kacskovics, L Hammarström: The porcine Ig delta gene: unique chimeric splicing of the first constant region domain in its heavy chain transcripts. *J Immunol*. **171**, 2003, 1312–1318.