In this chapter we examine the properties of antigens, the foreign material that actually triggers the immune system to respond. We first look at the properties that determine whether a foreign substance can act as an antigen. Then we examine antigens in more detail to determine what special features are recognized by the immune system. These special features tell us much about how the body recognizes invaders as well as about the structure of biological molecules. Finally, we briefly review some of the common antigens that we will encounter as we study immunology.

Since the function of the immune system is to protect the body against foreign invaders, it is fairly obvious that mechanisms must exist for cells to recognize these invaders. Somehow, the body must recognize a foreign substance in order for an immune response to be provoked. In this chapter, we discuss just what it is about foreign material that triggers immunity. Although we usually consider infectious microorganisms as the major threats to the body's integrity, it should be pointed out that we are exposed to a great variety of foreign material in everyday life and not all of it is threatening. Our most obvious exposure is to food. We consume quantities of foreign protein, carbohydrate, and fat. These foods are generally not a threat to the body and are not usually regarded as foreign. Likewise, large inert foreign bodies such as metal bone pins or plastic heart valves fail to provoke an immune response. We must therefore consider the features required of a foreign molecule in order for it to be recognized by the cells of the immune system, act as an antigen, and provoke an immune response.

ESSENTIAL FEATURES OF ANTIGENS

There are two major restrictions on antigenic molecules. First, and more important, the molecules must be recognized as foreign. Second, because of the processing antigens must undergo, there are physical and chemical limitations on the types of foreign molecules that can stimulate the immune system. The most effective antigens are large, rigid, chemically complex molecules that can be degraded fairly readily to soluble fragments that the immune system can recognize.

Factors That Influence Antigenicity (Fig. 4–1)

Molecular Size. In general, large molecules are better antigens than small molecules (Fig. 4–2). For example, hemocyanin, a very large protein from the blood of invertebrates (6.7×10^3 kDa), is a potent antigen. Serum albumin from other mammals (69 kDa) is a fairly good antigen but may also provoke

Hemocyanin - a potent ag.

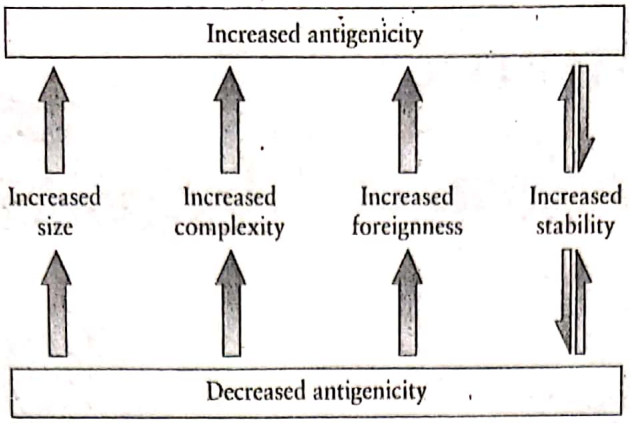


Figure 4–1 The major factors that determine antigenicity. Increased size, complexity, and foreignness all promote antigenicity. Physical stability has a more complex effect since very inert or very unstable molecules are poor antigens.

tolerance. The hormone angiotensin (1031 Da), however, is a poor antigen. The record for minimal antigenic size is held by p-azobenzene-arsenate trityrosine (750 Da), which has been used to provoke antibodies in guinea pigs and rabbits. Very small molecules may, however, bind to large proteins, and the resulting

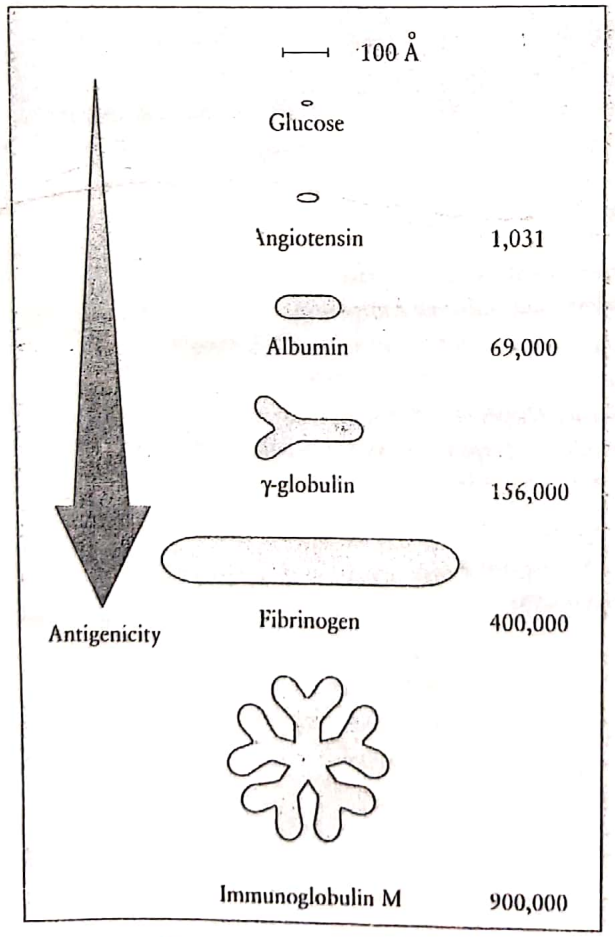


Figure 4–2 The relative sizes of some important antigens. Molecules the size of angiotensin or smaller are usually very poor antigens.

complex may then provoke an immune response. When this happens, these small molecules are called **haptens.**

Structural Stability. To recognize a molecule or part of a molecule as foreign, the cells of the immune system must recognize its specific shape. Consequently, highly flexible molecules that have no fixed shape are poor antigens. For example, gelatin, a protein well known for its structural instability (this is why it can wobble), is a poor antigen unless it is stabilized by the incorporation of tyrosine or tryptophan molecules, which cross-link the peptide chains. Similarly, flagellin, the major protein of bacterial flagella, is a structurally unstable, weak antigen. Its stability and hence its antigenicity are greatly enhanced by polymerization. Starch and other simple repetitive polysaccharides are poor antigens because they do not assume a stable configuration. For the same reason, proteins are better antigens than large, repeating polymers, such as the lipids, carbohydrates, and nucleic acids.

Degradability. The cells of the immune system recognize small molecular fragments and soluble antigens. If a molecule cannot be broken up or solubilized, then it cannot act as an antigen. For example, stainless steel pins and plastic joints are commonly implanted in the body without triggering an immune response. The lack of antigenicity of metals or large, inert organic polymers, such as the plastics, is due to their inertness. They cannot be fragmented and processed to a form suitable for triggering an immune response. Conversely, since immune responses are antigen driven, foreign molecules that are very rapidly destroyed on entering the body may not provide sufficient stable antigen fragments to stimulate an immune response.

Another example of the importance of fragmentation in antigen processing is seen when using copolymers of D–amino acids. D–amino acids do not occur naturally in mammals. Because mammalian enzymes cannot degrade them, they are metabolically inert. Peptides made from D–amino acids are therefore very poor antigens. If, however, a few short peptides consisting of L–amino acids are inserted into a D–amino acid polymer, the resulting molecule is a good antigen. The presence of the L–amino acids allows the peptide to be broken into fragments that can be recognized by the cells of the immune system.

Foreignness. The cells whose function is to respond to antigen (antigen-sensitive cells) are selected in such a way that they do not usually respond to normal body components. They will respond, however, to foreign molecules that differ even in minor respects from

those usually found within the body. The elimination of cells that react to normal body components and the subsequent development of tolerance to these components occur because these cells are exposed to self-antigens at an immature stage in their development (usually early in fetal life). Self-reactive immature cells exposed to self-antigens are killed. If antigen-sensitive cells are not exposed to an antigen when immature, tolerance to that antigen will not develop. For example, the sperm-forming cells in the testes are separated from the rest of the body by a tissue barrier. As a result, the cells of the immune system do not normally encounter sperm. These cells are therefore not tolerant to sperm antigens. If the tissue barrier is broken down by injury or infection, sperm antigen may reach the bloodstream, where the antigen-sensitive cells will regard it as foreign and mount an immune response against it. The development of antisperm antibodies is a common sequel to vasectomy as a result of leakage of sperm antigens into the tissues. On a smaller scale, mitochondria are not normally exposed to antigen-sensitive cells in the circulation. When extensive cell destruction occurs—as, for example, following a heart attack—mitochondria are exposed to the cells of the immune system and antimitochondrial antibodies may develop. These can be detected in serum several weeks later.

Foreign molecules differ in their ability to stimulate an immune response. This property is called **immunogenicity.** The immunogenicity of a molecule depends to a great extent on its degree of foreignness. The greater the difference between a foreign antigen and an animal's own antigens, the greater will be the intensity of the immune response. For example, a kidney graft from an identical twin will be readily accepted because its proteins are identical to those on the recipient's own kidney. A kidney graft from an unrelated human will be rejected in about two weeks unless drugs are used to control the rejection. A kidney grafted from a chimpanzee to a human will be rejected within a few hours despite the use of drugs.

EPITOPES

Complex foreign particles, such as bacteria, fungi, viruses, and foreign cells, readily provoke an immune response following injection. These particles are a complex mixture of proteins, glycoproteins, polysaccharides, lipopolysaccharides, lipids, and nucleoproteins. The response to such a foreign particle is therefore a mixture of many simultaneous immune responses against each of the foreign molecules.

A single large molecule such as a protein can also be shown to have regions against which the immune

responses are directed and with which antibodies will bind. These molecular sites that stimulate immune responses are termed **epitopes,** or **antigenic determinants** (Fig. 4–3). As with the intact molecule, the most intense immune responses are directed against epitopes that are most "foreign." As a result, some are much more immunogenic than others. Thus mice immunized with the enzyme lysozyme, obtained from chickens, preferentially respond to a single favored epitope, and the remainder of the molecule is virtually nonimmunogenic. Such epitopes are said to be **immunodominant.** In general, the number of epitopes on a molecule is directly related to its size, and there is usually about one epitope for each 5 kDa of a protein. For this reason, large molecules are usually more potent antigens than small molecules. When we describe a molecule as foreign, we are implying that it contains epitopes that are not found on self-antigens. The immune system recognizes and responds to such foreign epitopes.

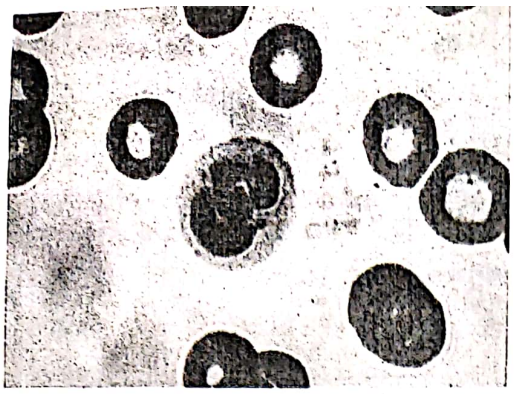


Figure 5–2 A neutrophil polymorphonuclear granulocyte in a blood smear (Wright-Giemsa stain; original magnification ×1400). *(From Bellanti, J. A. Immunology II. W. B. Saunders, Philadelphia, 1979. With permission.)*

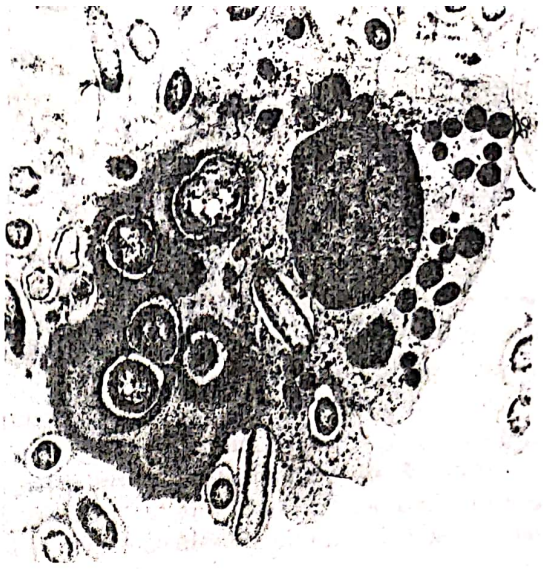


Figure 5–4 A transmission electron micrograph of a neutrophil phagocytosing bacteria. Several of the bacteria are already enclosed within phagosomes. *(Courtesy of Dr. Scott Linthicum.)*

blood cells) in blood, constituting about 60% to 75% of the blood leukocytes in humans.

Structure of Neutrophils

When suspended in blood, neutrophils are round cells about 12 μm in diameter (Fig. 5–2). They possess a finely granular cytoplasm, at the center of which is an irregular, sausage-like or segmented nucleus (Fig. 5–3). The chromatin in the nucleus is compacted and assumes this segmented shape since these cells are no longer able to divide. Electron microscopy shows that neutrophils contain two types of cytoplasmic granule. The primary granules are electron-dense structures that contain bactericidal enzymes such as myeloperoxidase and lysozyme; neutral proteases, such as elastase; and acid hydrolases, such as β-glucuronidase and cathepsin B. The secondary granules, which are not electron-dense, contain enzymes such as lysozyme and collagenase and the iron-binding protein lactoferrin. Mature neutrophils also have a small Golgi apparatus and some mitochondria but very few ribosomes or rough endoplasmic reticulum (Fig. 5–4). Most of the proteins necessary for their function are transcribed and translated during their development in the bone marrow. Thus they cannot synthesize large quantities of protein. Their cytoplasm also contains large amounts of glycogen. This glycogen is a source of glucose that can be used for anaerobic glycolysis. As a result, neutrophils can remain functional in damaged tissues where oxygen tension is low, such as at sites of bacterial invasion.

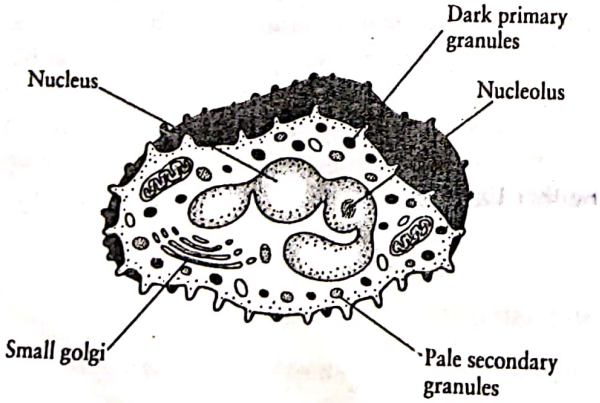


Figure 5–3 The major structural features of a neutrophil polymorphonuclear granulocyte. Note the highly irregular nucleus, indicating that this is not a dividing cell, as well as a lack of rough endoplasmic reticulum, suggesting that very little new protein synthesis will occur.

Functions of Neutrophils

Phagocytosis

The major function of neutrophils is the capture and destruction of foreign organisms through **phagocytosis.** Although a continuous process, phagocytosis can be divided into four discrete stages: chemotaxis, adherence, ingestion, and digestion (Fig. 5–5).

Chemotaxis

Chemotaxis is the directed movement of neutrophils under the influence of external chemical gradients. Thus neutrophils are attracted toward the source of certain chemicals. Since neutrophils can crawl but cannot swim, they must attach to a surface before they

ANTIGEN PROCESSING CELLS

Macrophages

Macrophages are the most accessible and best understood of the antigen-presenting cells. Their key properties have been described in the previous chapter. They are probably of greatest importance in processing antigen that has not been encountered previously by the body. Thus macrophages can phagocytose organisms in the absence of antibody for opsonization. They are probably of less importance when there are preexisting antibodies, since these greatly increase the efficiency of antigen processing by dendritic cells and B cells.

Dendritic Cells

Antigen processing by macrophages is inefficient, since much of the endocytosed antigen is destroyed by lysosomal proteases. An efficient alternative pathway of antigen presentation involves antigen uptake by a specialized population of mononuclear cells, collectively called dendritic cells.

Dendritic cells are located throughout the body, but especially in lymphoid organs. They have many long, filamentous cytoplasmic processes called dendrites (Fig. 8–2). They also have lobulated nuclei and a clear cytoplasm containing characteristic granules called Birbeck granules. All dendritic cells carry complement and antibody receptors on their surface but are poorly phagocytic. Several types of dendritic cells are found in the body. These include **Langerhans cells** located in the skin and interdigitating cells and follicular dendritic cells in lymphoid tissues.

Figure 8–2 A scanning electron micrograph of a dendritic cell from a guinea pig lymph node. Note its extensive array of dendrites that trap antigen (×4000).

In lymphoid tissues (e.g., spleen and lymph nodes), dendritic cells form an extensive interdigitating web that efficiently traps antigen while at the same time allowing cell interactions and movement. There are two populations of these cells, interdigitating cells in the T-cell areas (see Chapter 9 on the structure of lymphoid tissues) and follicular dendritic cells in the B-cell areas. Interdigitating cells are present in all lymphoid tissues, including the thymus. Follicular dendritic cells are confined to the follicles in the B-cell areas where they form clusters with lymphocytes.

Langerhans cells are dendritic cells found in the epidermis of the skin. They trap and present antigen that penetrates the skin. This includes topically applied antigen such as the resins of poison ivy or intradermally injected antigens such as mosquito saliva. As a result, Langerhans cells influence the development of skin allergies such as **delayed hypersensitivity** and **allergic contact dermatitis** (Chapter 30). Langerhans cells can leave the skin and colonize lymph nodes, where they become interdigitating cells. Veiled cells are the dendritic cells found in the lymph that flows from tissues into lymph nodes (efferent lymph). They may represent a transition form between Langerhans cells and interdigitating cells.

Dendritic cells present antigen in two ways. In an unprimed animal (i.e., an animal that has not previously been exposed to the antigen), antigen presentation is a passive process. The dendritic cells simply provide a surface on which antigen can be presented. In animals that have previously been exposed to an antigen and so possess antibodies, the antigen and antibody combine to form antibody–antigen complexes (also called **immune complexes**). Follicular dendritic cells in the B-cell areas take up these immune complexes on their surface and then shed them in round, beaded structures from their processes (Fig 8-3). These immune complex bodies (also called **iccosomes**) subsequently attach to B cells. The B cells ingest the antigen and, after processing, present it to

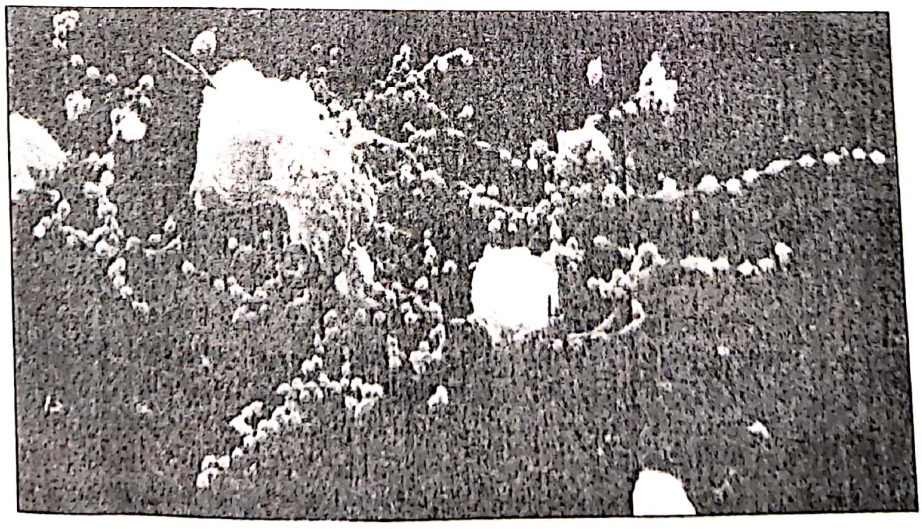


Figure 8–3 Scanning electron micrograph of a follicular dendritic cell with "beaded" dendrites. The beads, which are known to be coated with immune complexes, are termed "iccosomes" (immune-complex–coated bodies). The arrow indicates the cell body from which the dendrites emanate. L is a lymphocyte attached to the dendrites (original magnification ×3700). *(From Szakal, A.K., et al. J. Immunol., 134:1353–1354, 1985, © 1985 American Association of Immunologists. Reprinted with permission.)*

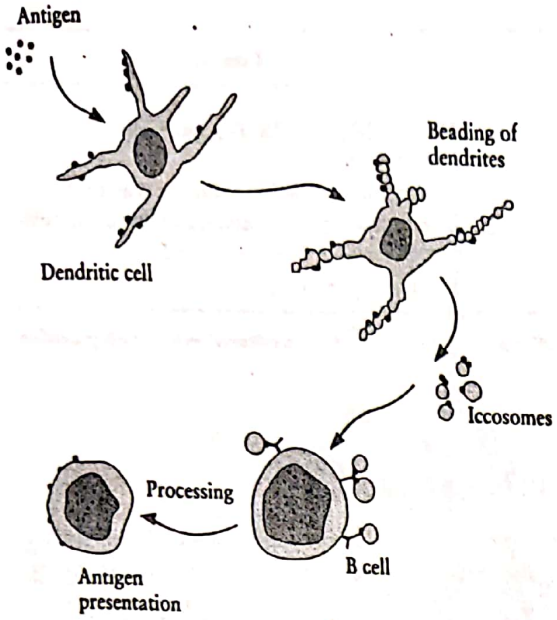


Figure 8–4 The suggested way in which iccosomes deliver antigen to B cells for processing.

antigen-sensitive T cells (Fig. 8–4). Dendritic cells can retain antigen on their surface for more than three months. Antigen processed by dendritic cells is a potent stimulant for T cells—about 10,000 times more efficient than unbound antigen.

B Cells

One type of lymphocyte called the B lymphocyte. B cell may also be an effective and important antigen-presenting cell. This is described in Chapter 14 but may be summarized here. B cells bind whole antigen molecules by means of their antigen receptors. They then ingest and process it and present the processed antigen, in association with specific antigen-presenting molecules to T cells. B cells are especially effective in presenting antigen to memory T cells and Th1 cells since they secrete interleukin-12. The importance of B cells as antigen-presenting cells can be demonstrated by showing that T-cell responses are seriously impaired in B-cell–deprived animals.

Other Antigen-Presenting Cells

Antigen may be presented to lymphocytes, albeit poorly, by many cell types. These include eosinophils, vascular endothelial cells, and skin keratinocytes. For example, vascular endothelial cells can also take up antigen, synthesize IL-1, and, under the influence of interferon, express antigen-molecules on their surface. Even skin keratinocytes can produce factors similar to IL-1, express antigen-presenting molecules, and present antigen to T cells.

THE MAJOR HISTOCOMPATIBILITY COMPLEX

Antigen processing requires not only the fragmentation of antigen molecules inside cells, but also the linkage of these fragments to appropriate antigen-presenting molecules. These antigen-presenting molecules are called **histocompatibility** or **MHC molecules.** They are in fact specialized receptor glycoproteins inherited in a gene complex called the **major histocompatibility complex (MHC).** The absolute need for antigens to be presented bound to MHC molecules is called **MHC restriction.**

All mammals possess a major histocompatibility complex in their genome. In humans the MHC is located on the short arm of chromosome 6; in mice it is found on chromosome 17. The human MHC contains about 3.5 megabases, which is two to three times larger than the mouse MHC and about the same size as the genome of the bacterium *Escherichia coli.*

The major histocompatibility complex contains three classes of genes (Fig. 8–5). Class I genes code for MHC molecules found on the surface of most nucleated cells (and on red cells in some species). The class I genes can be divided into those that are highly

MHC contains 3 classes of genes. Class I = highly polymorphic

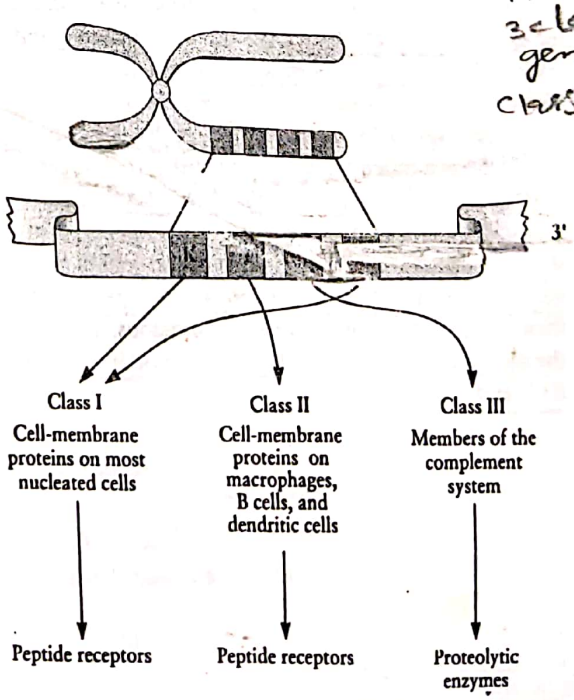


Figure 8–5 The three major classes of genes found within the mouse major histocompatibility complex and the functions of their gene products.

This chapter begins with a brief background of the antibodies found in serum and how they are classified as proteins known as immunoglobulins. Then the structure of the most important immunoglobulin, IgG, is examined. This includes the structural features of each of its peptide chains and domains. To understand how this structure was arrived at, we spend a little time describing the tumors of plasma cells called myelomas, since these were of immense importance in providing material for study. The use of immunoglobulin domains in the family of proteins called the immunoglobulin superfamily is also described. Then we examine each major class of immunoglobulin, in turn describing its structure and key features. We briefly review the variations in antibody structure found in different individuals as well as the structural variations in the immunoglobulins within an individual. The last part of the chapter deals with a slightly different topic, the strength of binding of antigen and antibody. This binding strength, or affinity, has important effects on diagnostic tests as well as on resistance to disease, and its measurement and significance are discussed here.

The protective factors produced by the immune responses were found in serum and called **antibodies** by Emil von Behring in 1890. In 1930, Heidelberger showed that antibodies were proteins by demonstrating that pneumococcal polysaccharide antigen, when added to antiserum so that it bound antibody, formed a precipitate that contained large amounts of protein.

PROPERTIES OF ANTIBODIES

Antibodies can be detected in many body fluids, for example, tears, respiratory tract mucus, saliva, intestinal contents, urine, and milk. They are present in highest concentrations and most easily obtained in large quantities from blood **serum.** It is important to point out, however, that antibodies also act as the antigen-binding receptors of B cells (BCR). This dual function of antibodies, as cell receptors and as soluble serum proteins, is in marked contrast to the TCRs, which are found only in the cell-bound form.

Because antibodies can be isolated in very large quantities from serum, it has been possible to study their structure in great detail. Indeed, unlike TCRs, antibodies can be readily sampled and their levels measured. For example, antibodies, like other serum proteins, can be characterized by their electrophoretic mobility.

Electrophoretic Mobility

Since the overall charge on any protein depends on its amino acid composition, a mixture of proteins may be fractionated by subjecting it to an electrical potential at a standard pH. This technique is known as **electrophoresis.** The positively charged protein molecules are attracted toward the cathode, the neutral molecules remain stationary, and the negatively charged molecules are attracted toward the anode. Each protein moves at a rate dependent on its net charge.

When serum is electrophoresed, it consistently separates into four major fractions. The most negatively charged fraction consists of a single protein called serum **albumin.** The other three major fractions are classified into α, β, and γ **globulins** according to their electrophoretic mobility. Antibody activity is mainly found in the fraction nearest the cathode, the γ globulins, although some antibodies are also found among the β globulins. Because antibody proteins are all globulins, they belong to a class of glycoproteins called **immunoglobulins.**

Overall Structure

Five distinct immunoglobulin **classes** (or **isotypes**) have been identified (Table 13–1). The major immunoglobulin in serum is immunoglobulin G (usually abbreviated to IgG), a molecule of 160 kDa. The second major immunoglobulin in serum is a large molecule of 900 kDa called immunoglobulin M (IgM). The third immunoglobulin class is immunoglobulin A (IgA). IgA, a molecule of 360 kDa, is found in such

Table 13–1 The Major Immunoglobulin Classes of Humans

	IgG	IgM	IgA	IgE	IgD
Molecular weight (kDa)	160	900	360	200	160
% Carbohydrate	3	12	7	12	12
Electrophoretic mobility	γ	β	β − γ	β − γ	γ
Heavy chain	γ	μ	α	ϵ	δ
Heavy chain domains	4	5	4	5	4
Subclasses	γ1, γ2, γ3, γ4	None	α1, α2	None	None
Half-life (days)	21	5	6	2	3

body secretions as saliva, milk, and intestinal fluid. Immunoglobulin D (IgD) (180 kDa) is mainly restricted to B-cell membranes but is present in very low concentrations in plasma. The fifth class, called immunoglobulin E (IgE), consists of molecules of 200 kDa that mediate allergic reactions.

STRUCTURE OF IMMUNOGLOBULINS

Immunoglobulin G

IgG, a glycoprotein of 160 kDa, is the predominant immunoglobulin class in serum. Its structure can serve as a model for the other immunoglobulins. IgG is a dimer of two disulfide-linked heterodimers. Each heterodimer contains a chain of 50 to 60 kDa called a heavy chain, disulfide-linked to a much smaller chain of about 25 kDa called a light chain (Fig. 13–1). The two heterodimers that are joined to form each IgG molecule are identical. Thus in total, an IgG molecule consists of four peptide chains—two heavy and two light.

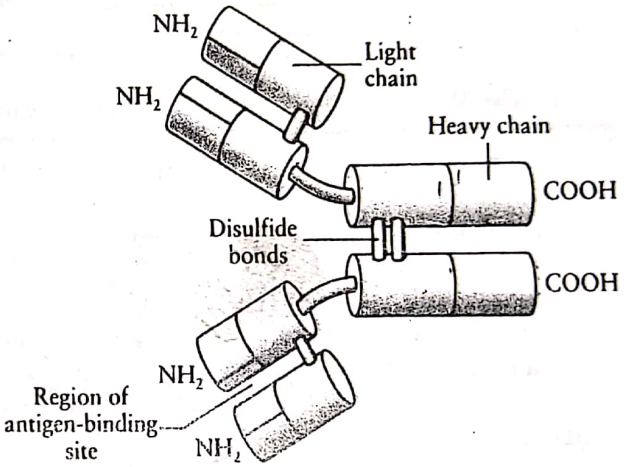


Figure 13–1 A simple model of an IgG molecule.

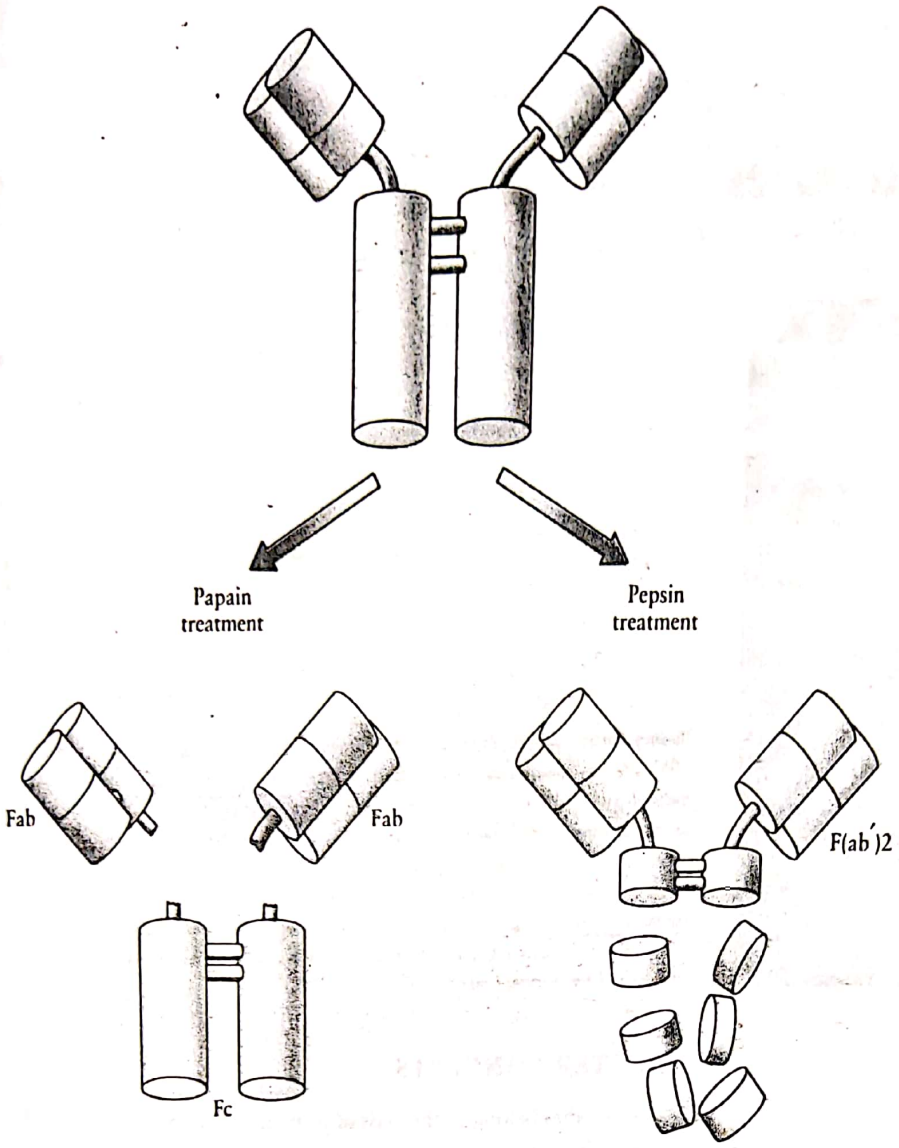


Figure 13–2 The effects of the proteolytic enzymes papain and pepsin on immunoglobulin G and the named fragments formed by this process.

If pure IgG is digested with the proteolytic enzyme papain, it splits into three approximately equal-sized fragments (Fig. 13–2). Two of these fragments retain the ability to bind antigen and therefore are called antigen-binding fragments, or **Fab fragments.** The third fragment obtained by papain treatment cannot bind antigen but is sometimes crystallizable. It is therefore called the **Fc fragment.** Other proteases such as pepsin may leave two Fab fragments joined together to produce a structure called F(ab′) 2.

When viewed with an electron microscope, IgG is seen to be Y-shaped (Fig. 13–3). These observations together with many others have shown that the complete IgG molecule has two binding sites for antigen, each located on one of the "arms" of the Y between a heavy and a light chain. (These are called the **Fab**

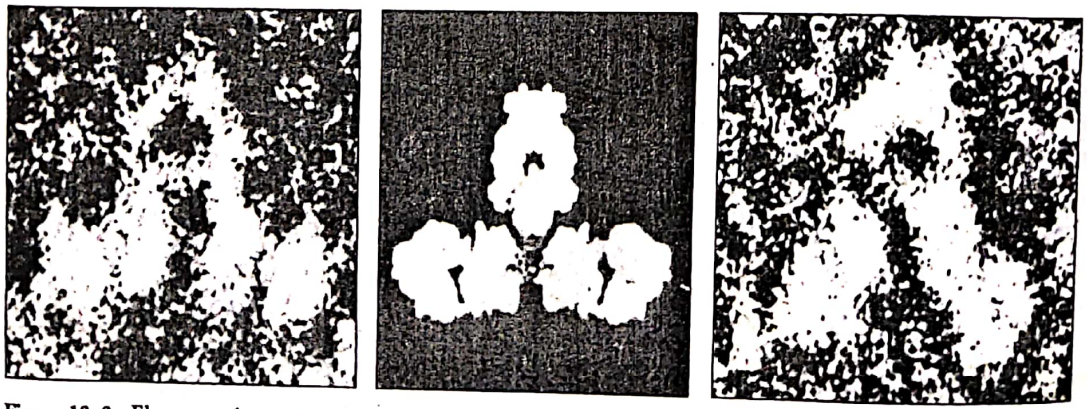


Figure 13–3 Electron micrographs of an IgG molecule. Compare the two electron micrographs with the computer-generated model in the center. This is rabbit IgG (original magnification ×2,042,500). *(From Roux, K.H., and Metzger, D.W. J. Exp. Med., 129:2548, 1982. With permission.)*

Table 13–2

	IgG1	IgG2	IgG3	IgG4
Chemical Properties				
% of total Ig in serum	65	24	7	4
Molecular weight (kDa)	146	146	170	146
Heavy chain	γ1	γ2	γ3	γ4
Inter-heavy-chain bonds	2	4	13	2
Biological Properties				
Complement activation	++++	++	++++	(+)
Half-life (Days)	23	23	8	23
FcR binding				
Neutrophils	+	+	+	+
Macrophages	+	−	+	−
NK cells	+	−	+	−
Placental passage	+	−	+	+
Skin sensitization	+	−	+	+

regions.) The "tail" of the Y forms a third region called the **Fc region.** The paired heavy chains extend the full length of the molecule. The light chains in contrast are found only in the arms of the Y in the Fab regions (see Fig. 13–2).

Types of Light Chains

Each immunoglobulin molecule has light chains of one of two types regardless of their class or antigen-binding ability. They are called κ (kappa) and λ (lambda) and are only about 35% homologous. The ratio of κ to λ chains varies between species. In humans, about 65% of immunoglobulin molecules have κ chains, and 35% have λ chains. Mice and rats have over 95% κ chains, while cattle and horses have 98% λ chains. Monkeys such as the rhesus monkey or the baboon have 50% of each.

Subclasses of IgG

Within the major immunoglobulin classes, there are structural variants known as **subclasses** that result from differences between heavy chains. Their amino

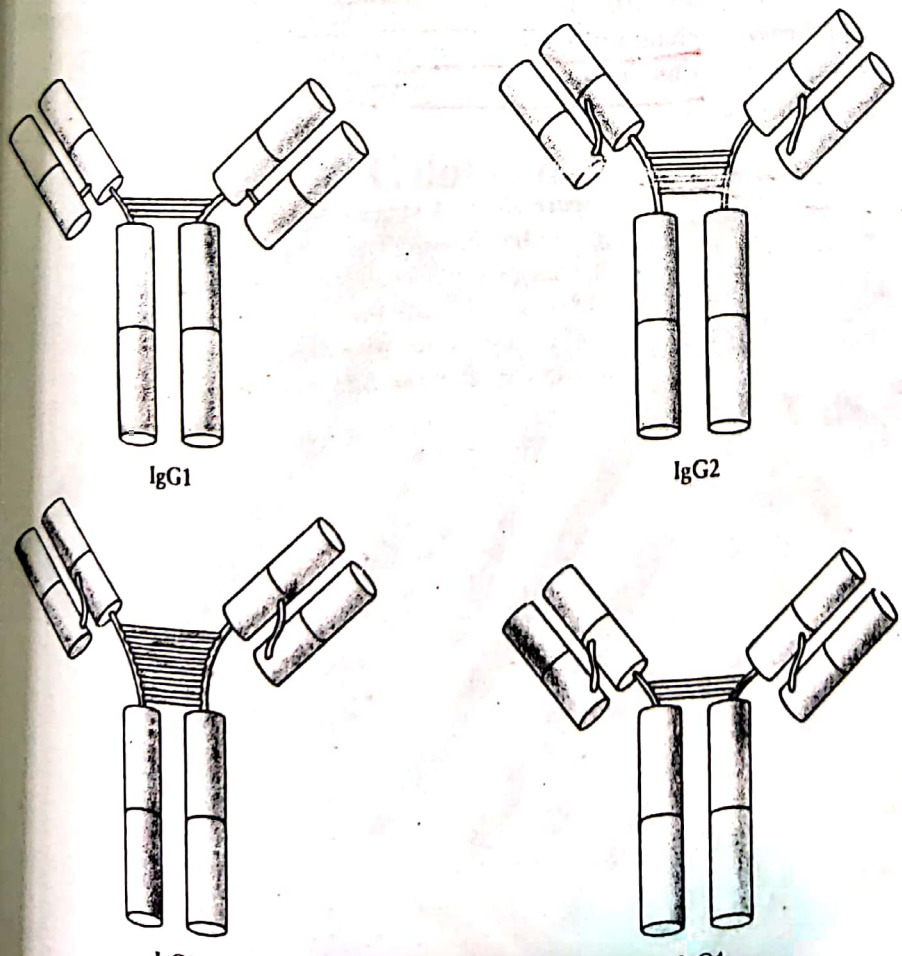


Figure 13–4 The basic structure of each of the human immunoglobulin G subclasses.

acid sequences and their antigenic differences are not great enough to constitute full classes, and they show a close overall relationship. Thus the four human IgG subclasses show greater than 90% sequence homology. Subclasses differ both in structure and in biological activities (Table 13–2). The four different IgG heavy chains in humans are designated γ1, γ2, γ3, and γ4. All normal persons possess all four subclasses. IgG molecules with γ1 heavy chains are known as IgG1, those with γ2 heavy chains are IgG2, and so on (Fig. 13–4). IgG3 has the shortest half-life, the lowest synthetic and highest catabolic rate, and an unusually high number of interchain disulfide bonds. Autoantibodies to clotting factors tend to belong to the IgG4 subclass, and autoantibodies to DNA tend to belong to the IgG1 and IgG3 subclasses. IgG4 is functionally monovalent and so will not precipitate or agglutinate antigen.

PRIMARY STRUCTURE OF IMMUNOGLOBULINS

The immunoglobulins found in serum are a mixture of molecules with antibody activity against a wide spectrum of epitopes. They represent a sample of the antibodies produced by that individual in response to a multitude of different antigenic stimuli. Because of this heterogeneity, it is not possible to use serum antibodies to analyze immunoglobulin structure in more than the general terms described previously.

However, immunoglobulins are secreted by **plasma cells.** Occasionally, a single plasma cell may become neoplastic, resulting in the growth of a clone of cancerous plasma cells that produces a single, absolutely homogeneous (**monoclonal**) immunoglobulin. As the tumor grows, large quantities of this monoclonal immunoglobulin are secreted into the bloodstream of affected individuals. Since a plasma cell tumor is called a **myeloma,** its monoclonal immunoglobulin product is called a myeloma protein (see page 208). Myeloma proteins may be purified and their structure analyzed in detail. One of the first tasks undertaken when myeloma proteins were recognized as monoclonal was to determine the sequence of amino acids in their light and heavy chains.

Light-Chain Sequences

Light chains each contain about 214 amino acids in two domains. If light chains from several different myelomas are studied, their amino acid sequences show important differences. In the C-terminal domain, the amino acid sequences are found to be almost identical. In contrast, the sequences in the N-terminal domain are very different in each light chain examined. For this reason, these two domains of a light chain are referred to as the **constant** (C_L) and **variable** (V_L) **regions,** respectively. This is, of course, a similar structure to that seen in TCR α and β chains where a variable domain is also linked to a constant domain.

Heavy-Chain Sequences

The heavy chains of IgG each consist of about 445 amino acids in four domains. In the center of the chain is an extended flexible hinge region. The sequence of amino acids in the N-terminal domain is different in each myeloma protein examined and thus constitutes a variable (V_H) region. The sequence in the other three domains located toward the C-terminus shows very few differences between different myeloma proteins and therefore forms a constant (C_H) region.

Variable Regions

When the amino acid sequences of many V regions from both light and heavy chains are examined in detail, two features emerge. First, the variation in amino acid sequence is largely restricted to three smaller regions within the entire variable region. These regions are therefore hypervariable. Between these **hypervariable regions** are the regions where the sequence is relatively [illegible] called **framework regions** (Fig. 13–5). We now know that the hypervariable regions are the regions that bind to an epitope. Their amino acid sequence determines the shape of the antigen-binding site (sometimes called the **paratope**), and they determine just which epitopes an immunoglobulin binds to (Fig. 13–6). They are therefore called **complementarity-determining regions** (CDRs). Each CDR is relatively short, consisting of six to [illegible] amino acids. Thus the CDRs of light chains include residues 24 to 34 (CDR1), 50 to 56 (CDR2), and 89 to 97 (CDR3). The CDRs on heavy chains include residues 31 to 35 (CDR1), 50 to 65 (CDR2), and 95 to 102 (CDR3) (Fig. 13–7).

The amino acid sequences of framework regions are not absolutely constant in that some minor variations in sequence do occur. Analysis of the variability within the framework regions reveals that variable regions can be classified into subgroups. In humans, there are four subgroups of V_κ regions, six subgroups of V_λ regions, and four subgroups of V_H regions. In the mouse, there are at least 27 and perhaps as many as 100 V_κ subgroups but only two V_λ subgroups and seven V_H subgroups. (The precise number of subgroups depends, of course, on the degree of homology used to define the subgroup.)

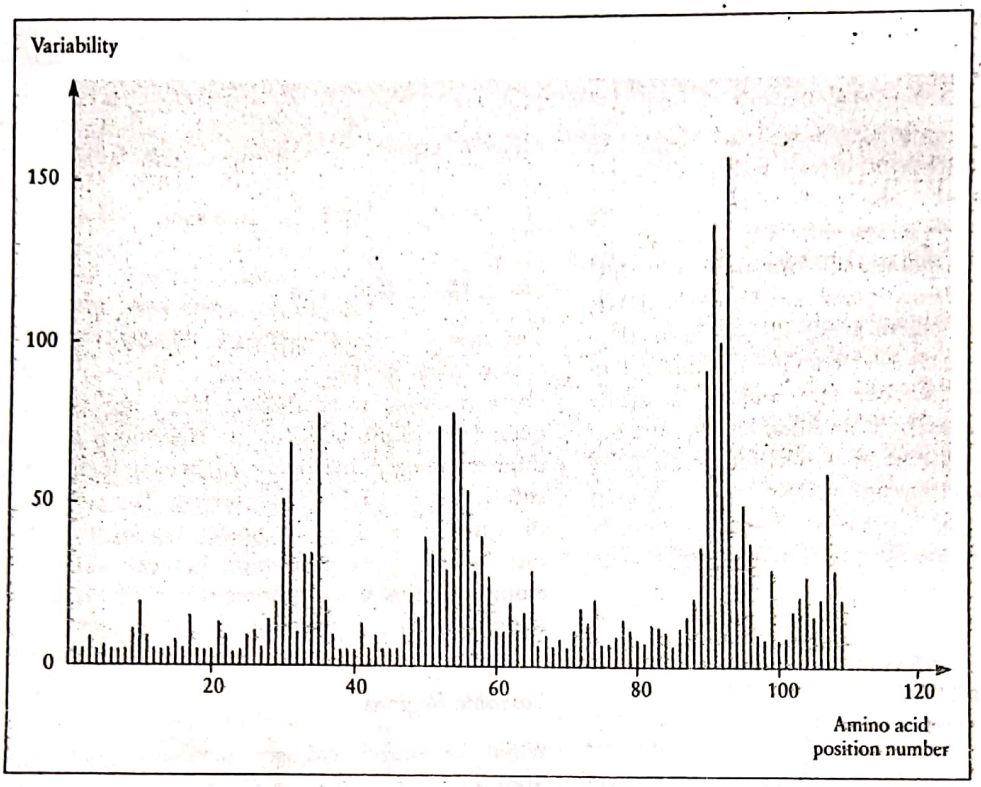
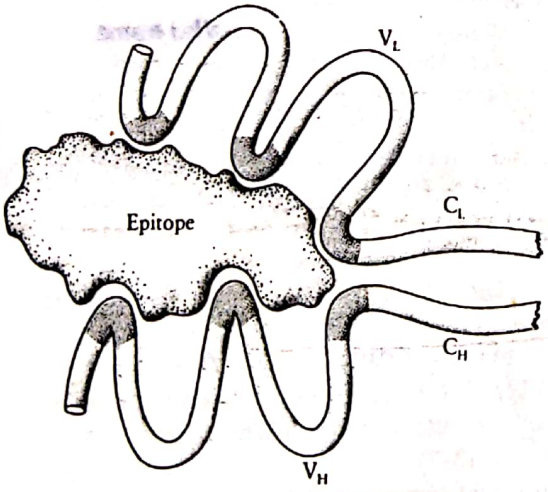


Figure 13–5 The variability of each amino acid position in an immunoglobulin light chain as shown by a Kabat-Wu plot. Note that there are three hypervariable regions, one of which is located close to the constant region around residues 95 to 100. This may be compared with a similar plot for the V region of the T cell antigen receptor (see Figure 11–4).



Constant Regions

The C_H region is made up of three almost identical domains, each of 110 amino acids. They are structurally similar to the C_L domain. It is believed that these domains result from the repeated duplication of a single primordial gene coding for a basic domain of 110 amino acids. Presumably, this gene duplicated at an early evolutionary stage to form a linked constant and

Figure 13–6 A schematic diagram showing the way in which light and heavy chains are folded so that the three complementarity determining regions in each chain form the walls of the antigen-binding groove.

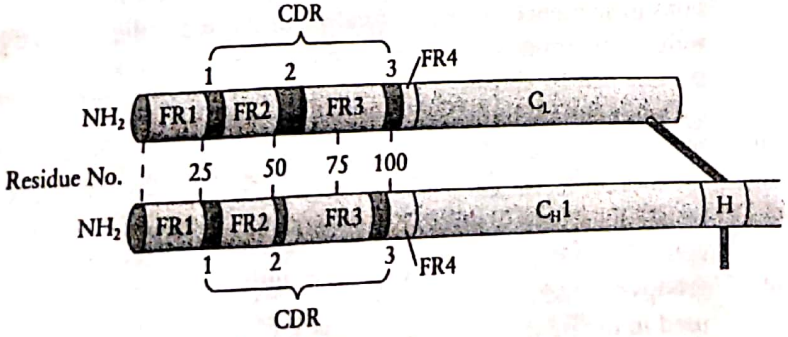


Figure 13–7 The location of the complementarity-determining, and framework regions in immunoglobulin light and heavy chains.

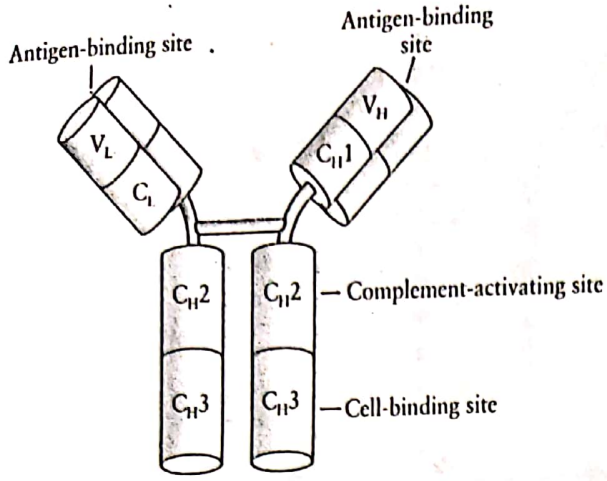


Figure 13–8 A model of an IgG molecule showing the domains and their biological activities.

variable domain and thus a primitive light chain (V_L and C_L). The constant domain was subsequently duplicated at least twice to form most of a heavy chain. One variable domain, one hinge region, and three constant domains make up a complete γ heavy chain. They are labeled from the N-terminal end, V_H, C_H1, H, C_H2, and C_H3. A similar arrangement is found in the α chains of IgA. In IgM and IgE heavy chains, an additional domain known as C_H4 is present. As a result, the Fc regions of IgM and IgE are larger than those of IgG or IgA.

Since heavy and light chains as well as both heavy chains are covalently linked, domains come together to form specialized regions within the immunoglobulin molecule. These paired domains provide a structure by which immunoglobulin molecules can exert their biological functions. Thus V_H and V_L form a paired domain that binds antigen, and C_H1 and C_L together act to stabilize the antigen-binding site. The paired C_H2 domains of IgG contain a site for the activation of the complement cascade (see Chapter 16) and a site that binds to receptors on phagocytic cells (Fig. 13–8). These domains also influence the catabolic rate of IgG. The structure of the heavy chain also regulates the transfer of IgG across the placenta to the fetus and antibody-mediated cellular cytotoxicity (Chapter 18), although these are probably due to the combined activities of several domains.

Hinge Region

On electron microscopy of IgG, it can be seen that the Fab regions are mobile and can swing freely around the center of the molecule as if they are hinged (Fig. 13–9). This hinge consists of about 12 amino acids located between the C_H1 and C_H2 domains. There is no homology between the hinge and other heavy-chain domains, and the sequence is unique for each immunoglobulin class and subclass. The hinge region contains many hydrophilic and proline residues (Fig. 13–10). The hydrophilic residues make the peptide chain unfold and thus make this region readily accessible to proteolytic enzymes. The proline residues

IgM & IgE have additional C_H4, hence larger Fc region.

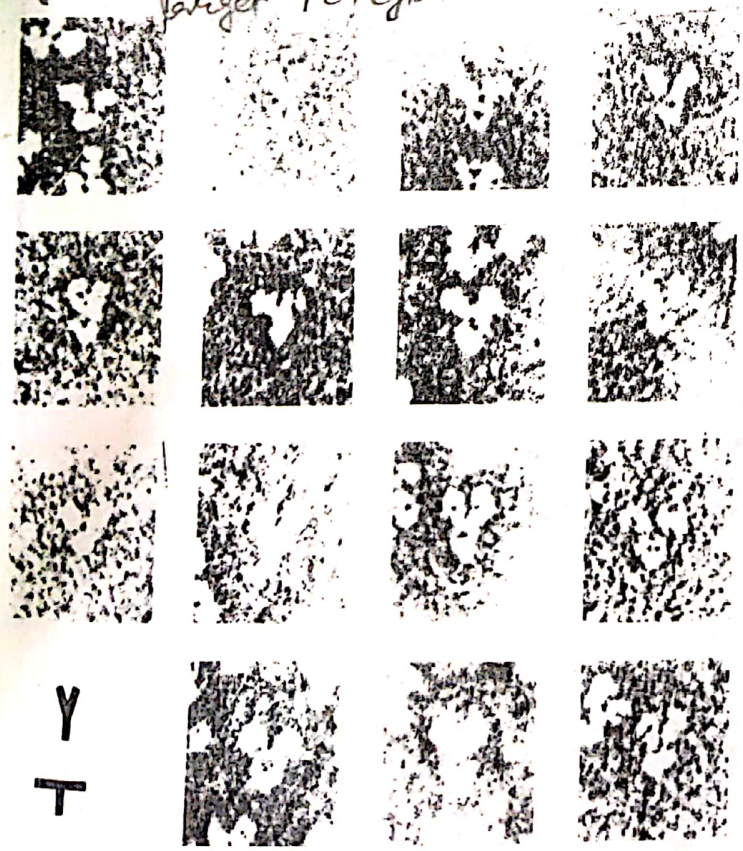


Figure 13–9 A series of electron micrographs of rabbit IgG showing how the Fab regions can move as a result of the flexibility of the hinge region, and a diagram showing the two extremes of the basic "Y" configuration. *(From Roux, K.H., and Metzger, D.W. J. Exp. Med., 129:2548, 1982. With permission.)*

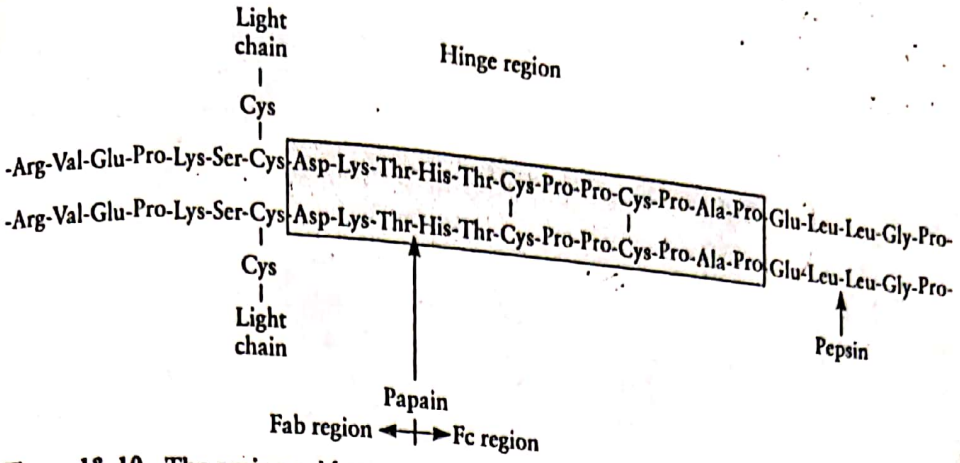


Figure 13–10 The amino-acid sequence within the hinge regions of the human IgG2 subclass. Note the large numbers of proline residues that confer flexibility and the cysteine residues that form interchain disulfide bridges. The diagram also shows the sites of pepsin and papain action.

make the chain flexible. This is the region where pepsin and papain act. This region also contains all the interchain disulfide bonds.

THE IMMUNOGLOBULIN SUPERFAMILY

Not only is the immunoglobulin domain the major structural unit of immunoglobulin constant regions, but individual immunoglobulin-like domains are key components of many other proteins involved in cellular interactions. These molecules form an **immunoglobulin superfamily.** A superfamily is a set of proteins that arise from a common ancestor and, as a result, share significant sequence homology.

An immunoglobulin domain consists of about 110 amino acids. Their sequence gives it a characteristic shape of two β-pleated sheets stabilized by an intrachain disulfide bond. The folding varies slightly between variable and constant domains. There are three β strands in one sheet and four in the other in a constant domain (Figs. 13–11 and 13–12). The β strands

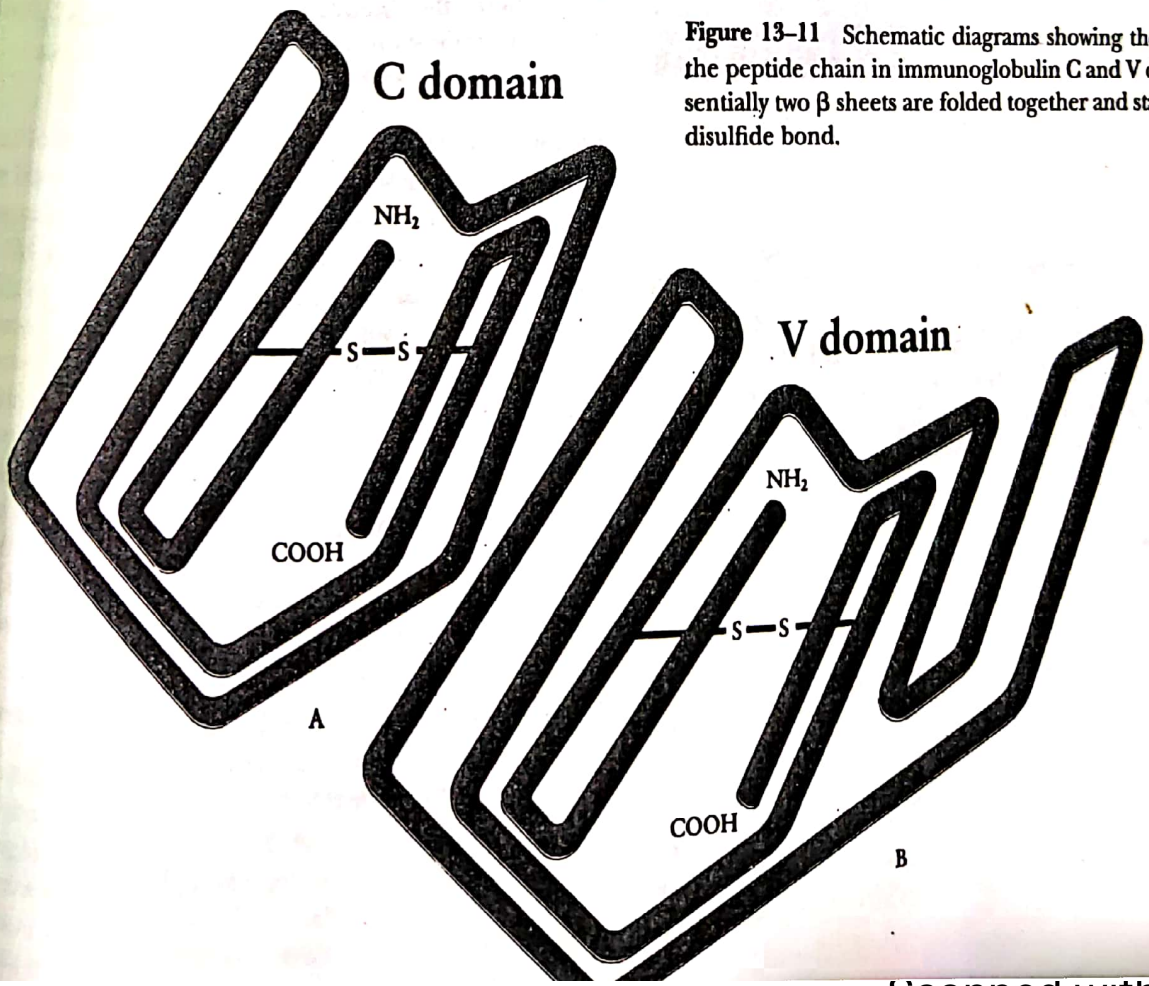


Figure 13–11 Schematic diagrams showing the folding of the peptide chain in immunoglobulin C and V domains. Essentially two β sheets are folded together and stabilized by a disulfide bond.

IMMUNOGLOBULIN CLASSES

Immunoglobulin G

Because IgG is the immunoglobulin class found in highest concentration in blood, it plays the major role in antibody-mediated defense mechanisms (Fig. 13–14). It has a molecular weight of about 160 kDa and

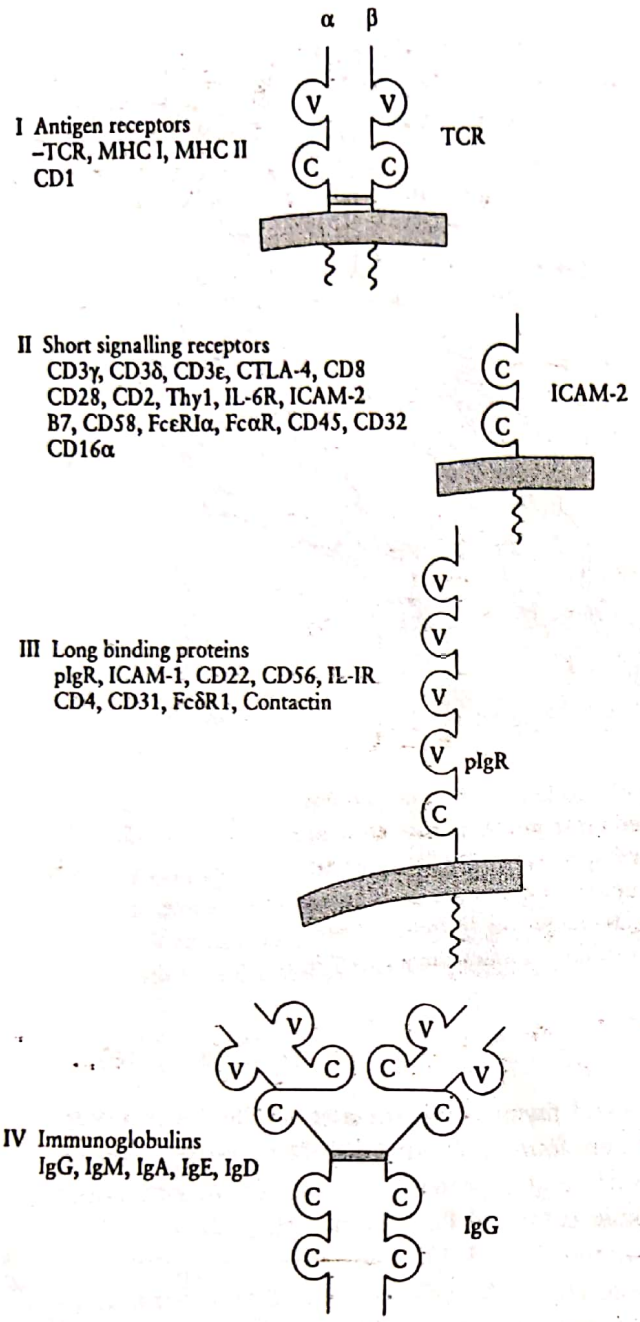


Figure 13–13 The members of the immunoglobulin superfamily. These fall into four major groups. (**a**) The antigen receptors, (**b**) the small receptors that contain one or two domains, (**c**) the large receptors that contain three or more domains, and (**d**) the immunoglobulins.

γ heavy chains. Because of its relatively small size, IgG can escape from blood vessels more easily than can the other immunoglobulin molecules. Therefore, it participates in the defense of tissue spaces and body surfaces. IgG can opsonize, agglutinate, and precipitate antigen (Chapter 17), but it can only activate the **classical complement pathway** when multiple IgG molecules have accumulated in a correct configuration on the antigen surface (Chapter 16).

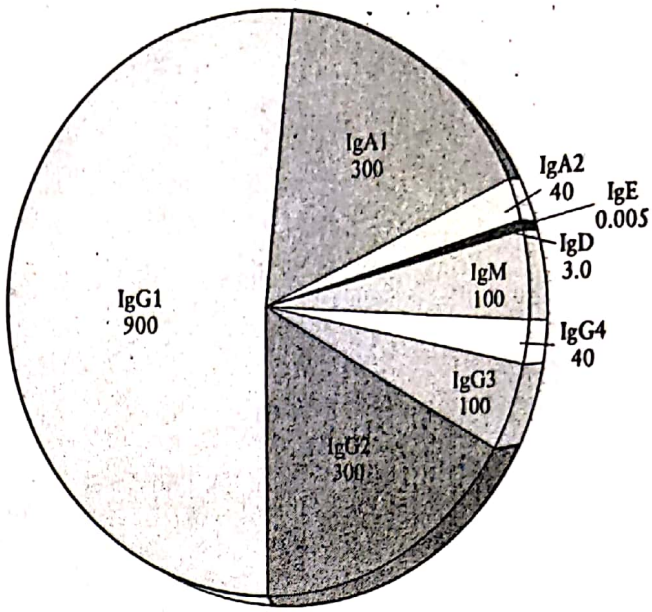


Figure 13–14 The immunoglobulins found in normal human serum. The numbers beside each class and subclass are average serum concentrations in mg/dl.

Immunoglobulin M

IgM is found in the second highest concentration after IgG in most mammalian serum; in humans, however, the IgA concentration in serum is slightly greater than that of IgM. Structurally, IgM is a polymer formed by five (rarely six) 180-kDa subunits (Fig. 13–15), so that its molecular weight is 900 kDa. Each subunit consists of two κ or two λ light chains and two μ heavy chains. Since each subunit possesses two antigen-binding sites, it might be anticipated that the valency of IgM for antigen would be 10. In practice, this valency is more commonly found to be 5 as a result of steric hindrance between antigen molecules. The μ chain has an additional fourth constant domain (C_H4) as well as an additional 20-amino-acid segment on its C-terminus, but it does not contain a hinge region. The site for complement activation by IgM is located on this C_H4 domain.

IgM **monomers** are linked by disulfide bonds in a circular fashion. A small cysteine-rich polypeptide called the **J chain** (15 kDa), coded for by a separate gene, binds two of the units to complete the circle. Since IgM molecules are normally secreted intact by plasma cells, the J chain must be considered an integral part of the molecule.

IgM is the major immunoglobulin class produced in a primary immune response. It is also produced during a secondary response, but this is commonly masked by the production of much larger quantities of IgG. In humans, for example, about 32 mg/kg of IgG is produced daily as opposed to 2 mg/kg of IgM. Although produced in relatively small quantities, when considered on a molar basis, IgM is more effi-

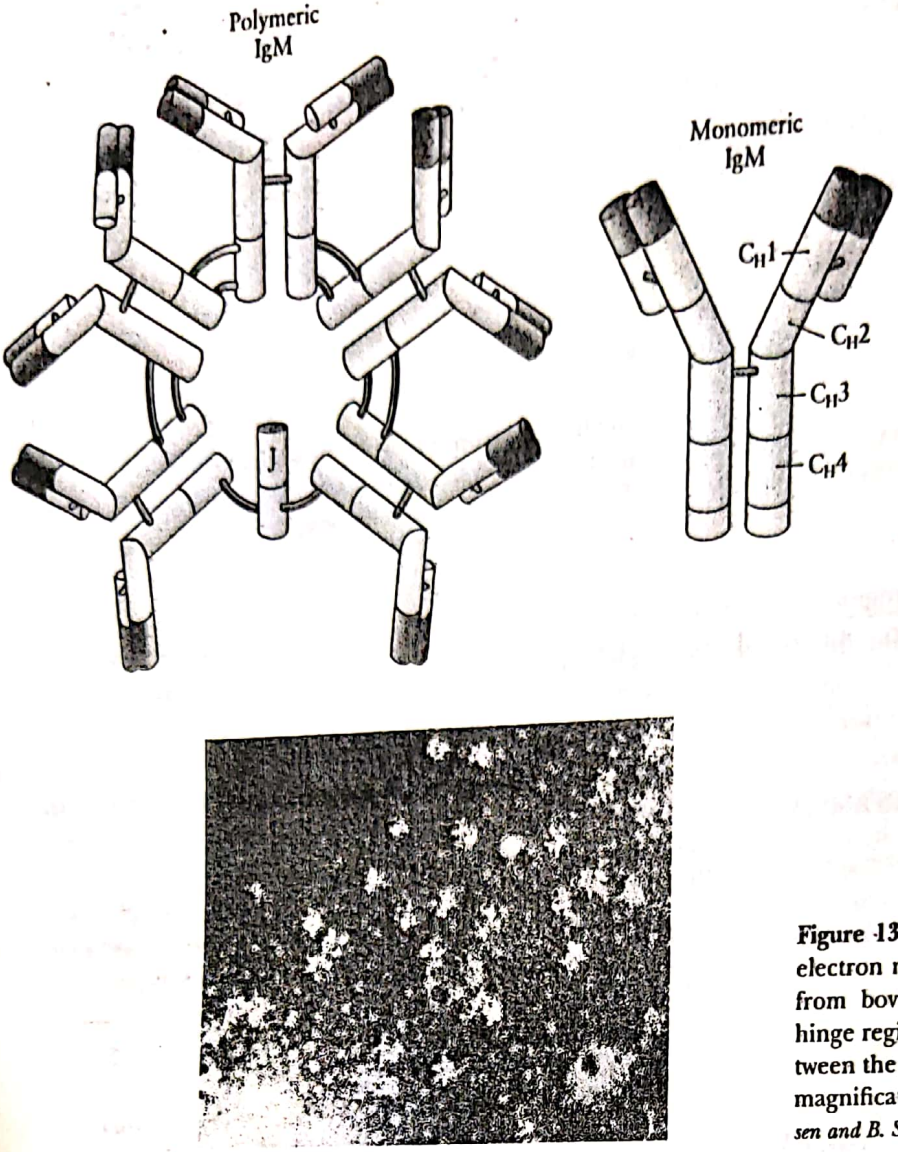


Figure 13–15 The structure of IgM and an electron micrograph of this immunoglobulin from bovine serum. Although IgM lacks a hinge region, the μ heavy chain is flexible between the C_H2 and C_H3 regions (approximate magnification ×240,000). *(Courtesy of Drs. K. Nielsen and B. Stemshorn.)*

cient than IgG at activation of the complement cascade, at opsonization, at virus neutralization, and at agglutination. Because of their large size, IgM immunoglobulins are confined to the blood and are therefore of little importance in conferring protection in tissue fluids or body secretions. When IgM acts as an antigen receptor on B-cell membranes (BCR), it is found only in the monomeric form (Chapter 14). This membrane-bound IgM also differs from the secreted form in that the C-terminus of the C_H4 domain is longer and contains a hydrophobic sequence that enables it to act as an integral membrane protein (Fig. 13–16).

Immunoglobulin A

Monomeric IgA has a molecular weight of 150 kDa. Each monomer has a typical four-chain structure consisting of paired κ or λ light chains and two α heavy chains. IgA, however, occurs naturally as a dimer. The two monomers are joined by a **J chain,** which links a Cα2 of one unit to the Cα3 of the other unit (Fig. 13–17). Higher polymers of IgA are occasionally found in serum. A membrane-bound form of IgA acts as a receptor on B cells. It differs from the secreted form in having a hydrophobic membrane-binding sequence at its C-terminus.

In many mammals, including humans, subclasses of IgA are recognized. In humans, differences in heavy-chain structure give rise to IgA1 and IgA2. There are also two variants of IgA2. One variant lacks disulfide bonds between the heavy and light chains, so that the molecule is held together only by noncovalent forces. Interchain bonds are present in the second IgA2 variant and in all IgA1 molecules. IgA is synthesized largely by plasma cells located on body surfaces. The IgA produced by the cells in the intestinal wall may either pass through epithelial cells into the intes-

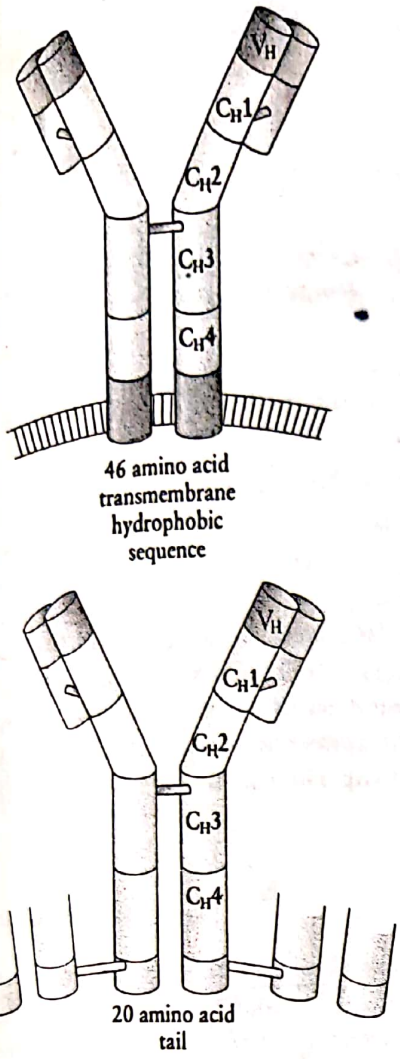


Figure 13–16 The differences in the heavy chains of cell-bound, and polymeric immunoglobulin M.

tinal lumen or, depending on the species, diffuse into the bloodstream. IgA within the bloodstream binds to hepatocytes and is carried through them into the bile. As the IgA is transported through intestinal epithelial cells or through hepatocytes, it is bound to a glycoprotein of 71 kDa known as **secretory component.** Secretory component binds covalently to IgA dimers to form a complex molecule called secretory IgA (SIgA). Secretory component protects IgA from digestion by intestinal proteolytic enzymes. Secretory IgA is of critical importance in protecting body surfaces against invading microorganisms since it is the major immunoglobulin in the intestinal, respiratory, and urogenital tracts; in milk; and in tears.

Immunoglobulin D

Immunoglobulin D molecules consist of two δ heavy chains and two κ or λ light chains. The molecular weight of IgD is about 170 kDa. IgD has only two domains in its heavy chains since it lacks a C_H2 domain. These two heavy-chain domains are separated by a long exposed hinge region (Fig. 13–18). IgD has no

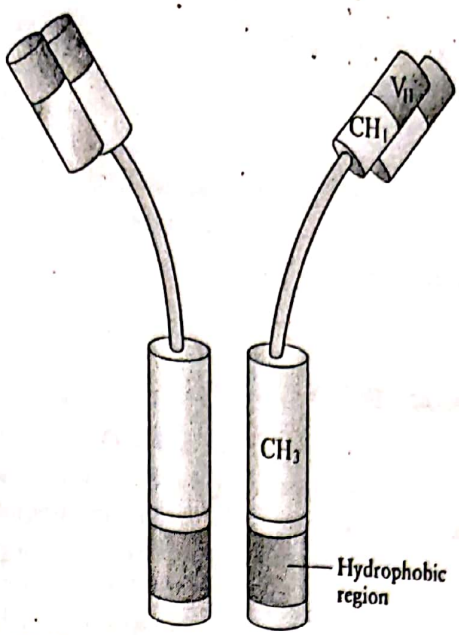


Figure 13–17 The structure of IgA. Secretory component protects IgA from proteolytic digestion.

interchain disulfide bonds between its heavy chains and, as a result, is unusually susceptible to proteolysis. Since proteases are generated when blood clots, IgD cannot be found in serum but is present in low concentrations in plasma. Like IgE, IgD is readily denatured by mild heat treatment. IgD antibodies with activity against thyroid tissue, insulin, penicillin, nuclear antigens, and diphtheria toxoid have been described. Plasma IgD levels are twice as high in smokers than in

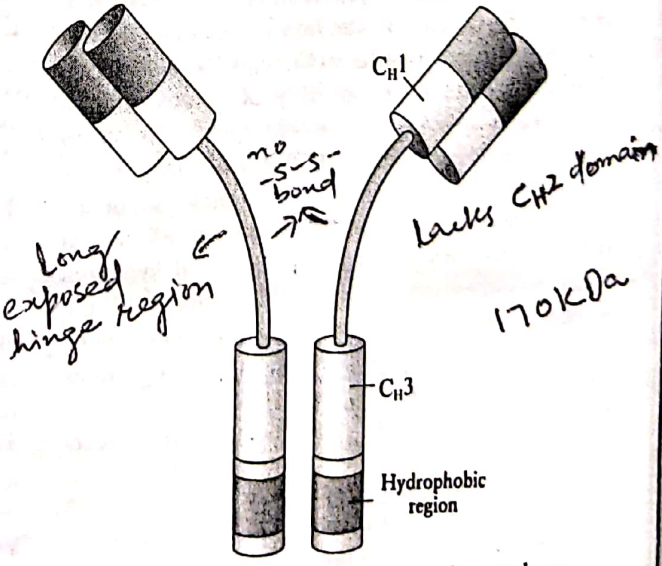


Figure 13–18 The structure of IgD showing the very long exposed hinge region that accounts for the extreme susceptibility of this molecule to proteolytic digestion.

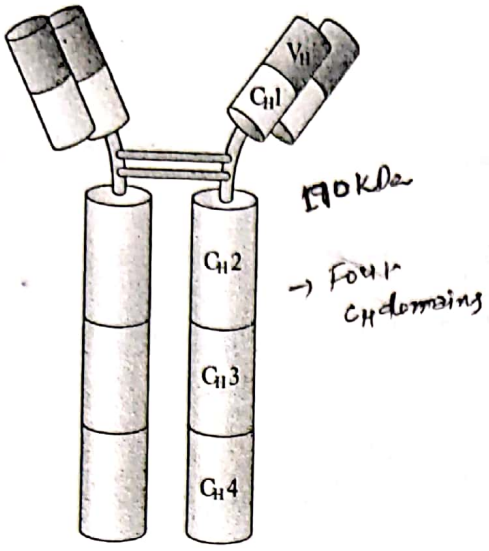


Figure 13–19 The structure of IgE. Note the presence of the additional domainthat permits the IgE to bind to Fc receptors on mast cells.

chain contains four constant domains, and as a result, IgE has a molecular weight of 190 kDa. IgE is found in extraordinarily low concentrations in the serum of unparasitized individuals, varying from 20 to 500 ng/ml. This is approximately 1/40,000 of the concentration of IgG. IgE therefore does not neutralize antigens directly. Nevertheless, IgE is important since its biological activities are greatly amplified by binding to receptors on mast cells and basophils. As a result, it mediates **allergies** (type I hypersensitivity reactions, Chapter 29) and is largely responsible for immunity to invading parasitic worms. The Fc region of IgE binds strongly to high-affinity receptors on mast cells and basophils and, together with antigen, mediates the release of inflammatory agents from these cells. The receptor-binding site is formed by the 12 N-terminal amino acids of the C_H3 domains.

nonsmokers. Nevertheless, extensive IgD production is not a common feature of conventional immune responses. IgD is primarily a B-cell antigen receptor (Chapter 14).

Immunoglobulin E

An IgE molecule contains two κ or λ light chains and two ε heavy chains (Fig. 13–19). The hinge region is replaced by a constant domain so that each heavy

SECONDARY STRUCTURE OF IMMUNOGLOBULINS

The three-dimensional structure of immunoglobulin molecules is relatively constant. Nevertheless, the presence of hypervariable regions within the variable regions ensures that there are significant differences in molecular shape at the antigen-binding site. A monomeric immunoglobulin molecule consists of three globular regions (two Fab regions and one Fc region) linked by a flexible hinge (Fig. 13–20). Each of these globular regions is made up of paired domains. Thus the Fab regions each consist of two interacting do-

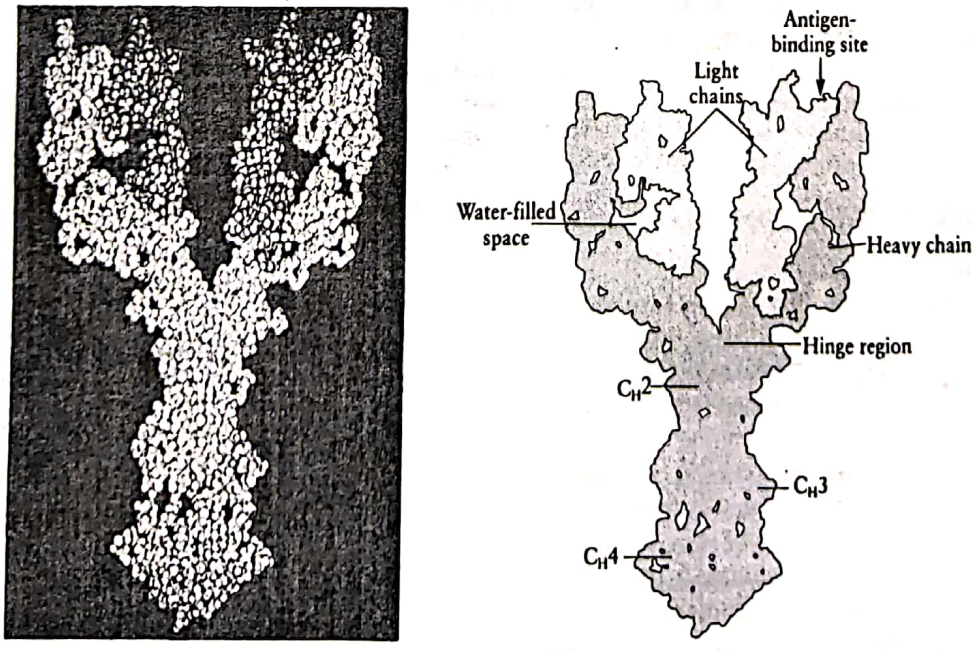


Figure 13–20 A molecular model of an IgE molecule showing the peptide backbone. The light chains are a darker shade than the heavy chains. The spaces within the molecule are normally filled with water. *(Courtesy of Dr. Scott Linthicum.)*

IMMUNOLOGY

- ✓ Immunology is the science that is concerned with immune response to foreign bodies.
- ✓ Immunity derived from the Latin word "immunis" meaning exempt is the ability of an organism to resist infections by pathogens and protection against foreign organisms.
- ✓ The cells, tissues, and organs that carry this function constitute the IMMUNE SYSTEM.
- ✓ Immunity is divided into two types: Innate immunity and Adaptive immunity.

INNATE IMMUNITY:

- ✓ Innate immunity is present since birth and acts against any foreign molecules and pathogens.
- ✓ It provides the first line of defense against pathogens. It is not specific to one pathogen but rather acts against all foreign molecules and pathogens.
- ✓ This type of immunity does not rely on previous exposure to a pathogen and response ifs functional since birth and has no memory.

Elements of innate immunity:

1. **Physical barrier**
   - ✓ They tend to prevent the entry of pathogens and are an organism's first line of defense against infection.
   - ✓ Skin and mucous membranes act as effective barriers against microorganisms.
   - ✓ Most of the organisms cannot penetrate the intact skin (exceptions damaged skin).
   - ✓ Acidic pH of sweat, sebaceous secretions, various fatty acids and hydrolytic enzymes such as lysozymes inhibit the growth of most microorganisms.
   - ✓ Mucous membranes present on the respiratory, gastrointestinal tract, conjunctiva of the eye protect aganist foreign microorganisms.
   - ✓ Several antimicrobial chemicals and phagocytic cells provide protection against pathogens.
2. **Chemical mediator**

   A variety of chemicals such as complement proteins, cytokines, pattern recognition molecules, acute-phase proteins, cationic peptides, enzymes like lyzosomes mediate protection against microbes.
   - ✓ Complement proteins are synthesized in the liver and circulate in the blood and extracellular fluid. Activation of the complement proteins in response to certain microorganisms results in a controlled enzymatic cascade, which targets the membrane of pathogenic organisms and leads to their destruction.
   - ✓ Cytokines are low molecular weight soluble proteins. It includes monokines, lymphokines, interleukin and interferons. Interferons are made by cells in response to virus infection. Chemokines are small, positively charged proteins that guide the migrations of various types of WBC's.
   - ✓ Other chemical mediators include soluble pattern recognition molecules like mannose-binding lectin (MBL), C-reactive protein (CRP) which bind to the microbial surface and promote their opsonization.
   - ✓ Acute phase proteins are a heterogeneous group of plasma proteins mainly produced in the liver as a result of microbial stimulus e.g. CRP, serum amyloid protein (SAA) and MBP. These proteins maximize the activation of the complement system and opsonization of the invading microbes.
3. **Cellular defenses**
   - ✓ Specialized cell types like neutrophils, macrophages, monocytes, natural killer cells and dendritic cells participate in innate host defense mechanisms. Phagocytosis (ingestion of invading foreign particles such as bacteria) is a fundamental protective mechanism carries out by these cell types. It is enhanced

by a variety of factors referred to as opsonins (consists of antibodies and various serum components of the complement).

4. **Inflammatory barriers**
   - ✓ Inflammation is an important nonspecific defense reaction to cell injury. Inflammatory response is mediated by a variety of signaling molecules.
   - ✓ Tissue damage caused by a wound or by an invading pathogenic microorganism induces a complex sequence of events collectively known as the **inflammatory response.**
   - ✓ The cardinal signs of inflammation reflect the three major events of an inflammatory response: Vasodilation (an increase in the diameter of blood vessels), an increase in capillary permeability and influx of phagocytes from the capillaries into the tissues.
   - ✓ One of the principal mediators of the inflammatory response is **histamine,** a chemical released by a variety of cells in response to tissue injury. Histamine binds to receptors on nearby capillaries and venules, causing vasodilation and increased permeability.
   - ✓ Small peptides called **kinins,** are normally present in blood plasma in an inactive form. Tissue injury activates these peptides, which then cause vasodilation and increased permeability of capillaries. A particular kinin, called bradykinin, also stimulates pain receptors in the skin.

ADAPTIVE IMMUNITY:

- ✓ Adaptive immunity is capable of recognizing and selectively eliminating specific foreign microorganisms and molecules (i.e., foreign antigens). Unlike innate immune responses, adaptive immune responses are not the same in all members of a species but are reactions to specific antigenic challenges.
- ✓ Adaptive immunity displays four characteristic attributes:
  1. Antigenic specificity: It is the ability to discriminate among different epitopes/ antigens.
  2. Diversity: The ability to respond to different epitopes even if the individual has not previously encountered them.
  3. Immunologic memory: It is the ability to recall previous contact with a foreign molecule and respond to it in a learned manner-i.e. with a more rapid and larger response.
  4. Self/non-self recognition: It is the ability to recognize and respond to molecules that are foreign and to avoid making a response to those molecules that are self (self tolerant).

Difference between innate and adaptive immune response

Attribute	**Innate immune response**	**Adaptive immune response**
Specificity	Antigen non-specific	Antigen specific
Response time	Rapid response(hours)	Slow response(days)
Diversity	limited	Very high
Memory	Absent or low	Present
Major cell types	Phagocytes, NK cells and others	T and B-cells, APS(antigen presenting cells
Distribution	Found in invertebrates and vertebrates	Found only in jawed vertebrates

- ✓ Adaptive immunity maybe passive or active. Active immunity is induced by natural exposure to a pathogen (natural) or by vaccination (artificial). In this kind of immunity a person develops his own immune response to a microbe.
- ✓ Immunity resulting from the transfer of antibodies or immune cells from an immune to a non-immune individual is known as passive immunity. It maybe natural (involves the transfer of antibodies from one host to another) or artificially (when antibodies or lymphocytes that have been produced outside the host are introduced into the host) acquired).

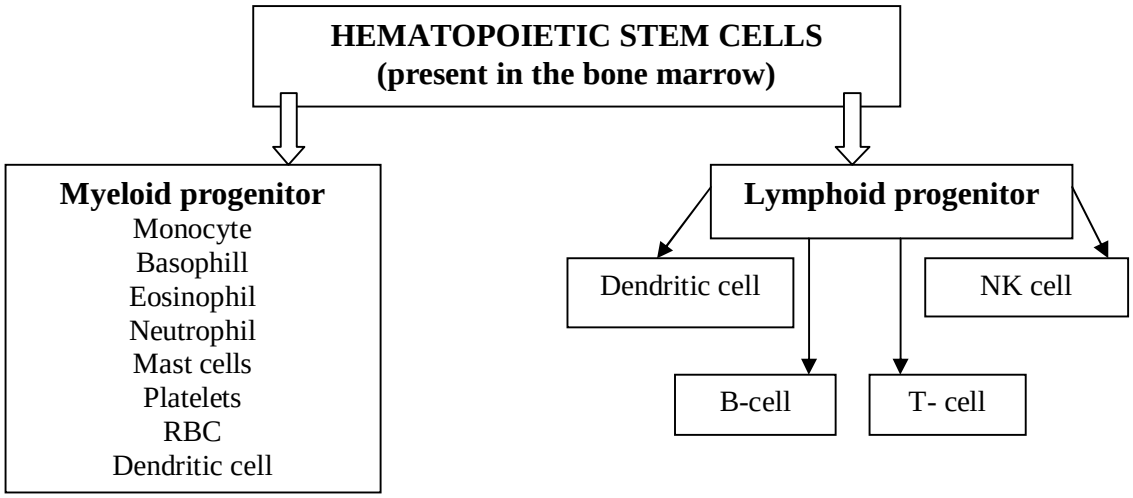
Difference between innate and adaptive immune response

Active Immunity	Passive Immunity
Exposure to antigen	No exposure to antigen
Immunity achieved by injecting antigens	Immunity achieved by injecting Ab or Ag reactive T-cells
Activation immune system	No immune system activation
Immune state develops over a period of weeks	Immunity develops immediately
Immunological memory develops	No immunological memory develops

- ✓ There are two branches of acquired immunity one is mediated by **B-cells** and circulating antibodies, a form of immunity which is referred to as **humoral immunity(** humor- body fluids)
- ✓ The other is mediated by **T-cells,** which do not synthesize antibodies but instead synthesize and release various cytokines that affect other cells. This is termed as **cellular or cell-mediated immunity**.
- ✓ In humoral immunity, B-cells (B lymphocytes) synthesize and secrete antibodies with specificity against the foreign substance.
- ✓ The T-cells (T- lymphocytes) which also exhibit specificity against the foreign substance do not make antibodies but perform various effector functions when APC's bring antigens into the secondary lymphoid organs.

CELLS OF THE IMMUNE SYSTEM

- ✓ The immune system is a defensive system in a host which constitutes of widely distributed cells, tissues and organs that recognize foreign substances and microorganisms and act to destroy them.
- ✓ The main cells that are responsible for immunity are the leukocytes/WBC's.
- ✓ Leukocytes arise from hematopoietic stem cell. It is a multipotent cell and gives rise to **lymphoid progenitor cell** or **myeloid progenitor cell**.



Lymphoid progenitor

- ✓ Lymphocytes are mononuclear leukocytes which constitute 20% - 40% of total WBC's. They occur in large numbers in the blood and lymph and lymphoid organs such as the thymus, lymph nodes, spleen and appendix.
- ✓ Lymphocytes are of three types:
  1. B-lymphocytes or B-cells
  2. T-lymphocytes or T-cells
  3. Natural killer cells (NK)

B-Lymphocytes:

- ✓ B-Lymphocyte matures in the bone marrow and expresses membrane bound antibody. After it comes in contact with the antigen it differentiates into the antibody-secreting **plasma cells** and **memory cells.** B-cells also serve as antigen presenting cells (APCs).
- ✓ Memory B cells have a longer life span than naive cells, and they express the same membrane-bound antibody as their parent B cell. Plasma cells produce the antibody in a form that can be secreted and have little or no membrane-bound antibody.

Properties of B-cell:

Origin	Bone marrow
Maturation	Bone marrow(Bursa of Fabricius in bird)
Expression of Ag receptor	Bone marrow
Differentiation	In lymphoid tissue
Surface immunoglobulin	Present
Immunity	Humoral
Distribution	Spleen, lymph nodes, Bone marrow and others.
Secretory product	Antibodies and cytokines
Complement receptors	Present

T-Lymphocytes:

- ✓ T-lymphocytes arise in the bone marrow but unlike B-cells the T-cells migrate to the thymus gland to mature. During its maturation the T-cell expresses a unique antigen-binding molecule, called the T-cell receptor on the membrane.
- ✓ Unlike membrane-bound antibodies on B cells, which can recognize antigen alone, T-cell receptors can recognize only antigen that is bound to cell-membrane proteins called **Major Histocompatibility Complex (MHC) molecules.** MHC molecules that function in this recognition event, which is termed "antigen presentation," are polymorphic (genetically diverse) glycoproteins found on cell membranes.

- ✓ T-cells do not make any antibodies but perform effector functions when APC's bring antigens into the secondary lymphoid organ. T-cells help in removing APC's, cancer cells, virus infected cells etc.
- ✓ T-cells express distinct membrane molecules. On the basis of the presence of one or the other of two membrane molecules, $CD4^+$ and $CD8^+$, there are two sub population of T-cells- **T helper cells T_H** (carries $CD4^+$ membrane glycoprotein on their surfaces) and **T cytotoxic cells T_C** (carriers $CD8^+$ membrane glycoprotein on their surfaces).
- ✓ The ratio of $CD4^+$ and $CD8^+$ T-cells is approximately 2:1 in normal human peripheral blood, but it may be altered by immunodeficiency and autoimmune diseases.
- ✓ Cytotoxic T-cells provide protection against intracellular pathogens such as viruses and bacteria that multiply in the host cell cytoplasm, where they sheltered from attack by antibodies. They provide this protection by killing the infected cell before the microbes can proliferate and escape from the infected cell to infect the neighboring cells.
- ✓ Helper T cells are crucial for defense against both intracellular and extracellular pathogens. They help stimulate B-cells to make antibodies that inactivate and eliminate extracellular pathogens and their toxic products.

Properties of T-cell

Origin	Bone marrow
Maturation	Thymus
Expression of Ag receptor	Thymus
Differentiation	In lymphoid tissue
Surface immunoglobulin	Absent
Immunity	Cell mediated and Humoral
Secretory product	Cytokines
Complement receptors	TCR on membrane

Antigen-presenting cells

- ✓ Activation of both the humoral and cell-mediated branches of the immune system requires cytokines produced by T_H cells. It is essential that activation of T_H cells themselves be carefully regulated, because an inappropriate T-cell response to self-components can have fatal autoimmune consequences.
- ✓ To ensure carefully regulated activation of TH cells, they can recognize only antigen that is displayed together with class MHC II molecules on the surface of antigen-presenting cells (APCs).
- ✓ These specialized cells, which include macrophages, B lymphocytes, and dendritic cells, are distinguished by two properties: (1) they express class II MHC molecules on their membranes, and (2) they are able to deliver a co-stimulatory signal that is necessary for T_H-cell activation.
- ✓ Antigen-presenting cells first internalize antigen, either by phagocytosis or by endocytosis, and then display a part of that antigen on their membrane bound to a class II MHC molecule. The T_H cell recognizes and interacts with the antigen–class II MHC molecule complex on the membrane of the antigen-presenting cell. An additional co-stimulatory signal is then produced by the antigen-presenting cell, leading to activation of the T_H cell.

Natural Killer Cells:

- ✓ NK cells are a class of lymphocytes that are distinct from cytotoxic T-cells. NK cells play a role in destroying cells infected with intracellular pathogens. They constitute 5-10% of lymphocyte population.
- ✓ NK cells destroy the target cell by releasing biologically potent molecules. They resemble T_c cells in their ability to destroy infected cells.
- ✓ They are capable of destroying malignant and virus infected cells without previous exposure or contact with the foreign antigen. Chediak-Higashi syndrome an autosomal recessive disorder is associated with a lack of NK cells.

Dendritic cells:

- ✓ Dendritic cells are bone marrow derived cells that arise for both the myeloid and lymphoid lineages. The myeloid pathway that gives rise to monocyte/macrophage cell type also gives rise to dendritic cells.
- ✓ Dendritic cells acquire antigen by phagocytosis; the antigen is processed, and mature dendritic cells present it to T_H cells.
- ✓ Dendritic cells are classifies into four types:
  1. Langerhans cells
  2. Interstitial dendritic cells
  3. Myeloid dendritic cells
  4. Lymphoid dendritic cells

Myeloid progenitor

- ✓ Cells that arise from a common myeloid progenitor include RBC's as well as various types of WBC's (such as granulocytes, monocytes, macrophages etc).

Granulocytes:

- ✓ Granulocytes have irregular shaped nuclei with 2-5 lobes and are often called polymorphonuclear leukocytes. Their cytoplasmic matrix has granules that contain reactive substances that kill microorganisms and enhance inflammation.
- ✓ There are three types:
  1. Basophils
  2. Eosinophils
  3. Neutrophils
- ✓ *Basophils* have a lobed nucleus and stain withat basic dye methylene blue. It comprises less than 1% of total WBC's. They are non-phagocytic, release substances that cause an allergic response. These molecules include histamine, prostaglandins, serotonin and leukotrienes. Basophils (and mast cells) possess high affinity receptors for one type of antibody known as IgE.
- ✓ *Mast cell* precursors are formed in the bone marrow and released into the blood in an undifferentiated state, until they reach the tissues. They have a large number of cytoplasmic granules containing histamine. Mast cells and basophils play role in allergic responses.
- ✓ *Neutrophils* have a multilobed nucleus and a granulated cytoplasm that stain with acidic and basic dyes. They (constitute about 50%-70% of the circulating WBC's) are produced by haemopoiesis in the bone marrow.

- ✓ Neutrophils are released into the peripheral blood and circulate for 7-10 hours before migrating into the tissues, where they have a life span of only a few days. Just like macrophages, they are active phagocytic cells.
- ✓ *Eosinophils* have a bilobed nucleus and stain with the acidic dye eosin. They comprise 2-5% of WBC's and are motile phagocytic cells that can migrate from the blood into the tissue space. Their role is important in the defense against protozoans and helminth parasites, mainly by releasing cationic peptides and reactive oxygen intermediates into the extracellular fluid.

Monocytes:

- ✓ Monocytes are mononuclear phagocytic leukocytes that circulate briefly in the blood stream before migrating into the tissues where they turn into macrophages or dendritic cells. Monocytes circulate in the blood stream for about 8 hours during which they enlarge, and then they migrate into the tissues and differentiate into specific tissue macrophages or dendritic cells.

Macrophages:

- ✓ Macrophages are phagocyte derived from blood monocytes. Monocytes that migrate into tissues in response to infection can differentiate into specific tissue macrophages. The monocyte is a small spherical cell with few projections, abundant cytoplasm and many granules.
- ✓ Following migration of monocytes from blood to various tissues they undergo further differentiation into a variety of histologic form all of which play a role in phagocytosis, including: Kupffer cells in the liver, alveolar macrophages in the lung, spleen macrophages in the white pulp, peritoneal macrophages, osteoclasts in the bone, mesangial cells in the kidney and microglial cells in the central nervous tissue.

Organs involved in Immune response:

- ✓ The maturation, differentiation and proliferation of B and T- lymphocytes take place in the lymphatic organs. They are generally divided into two categories: Primary and Secondary lymphoid organs.

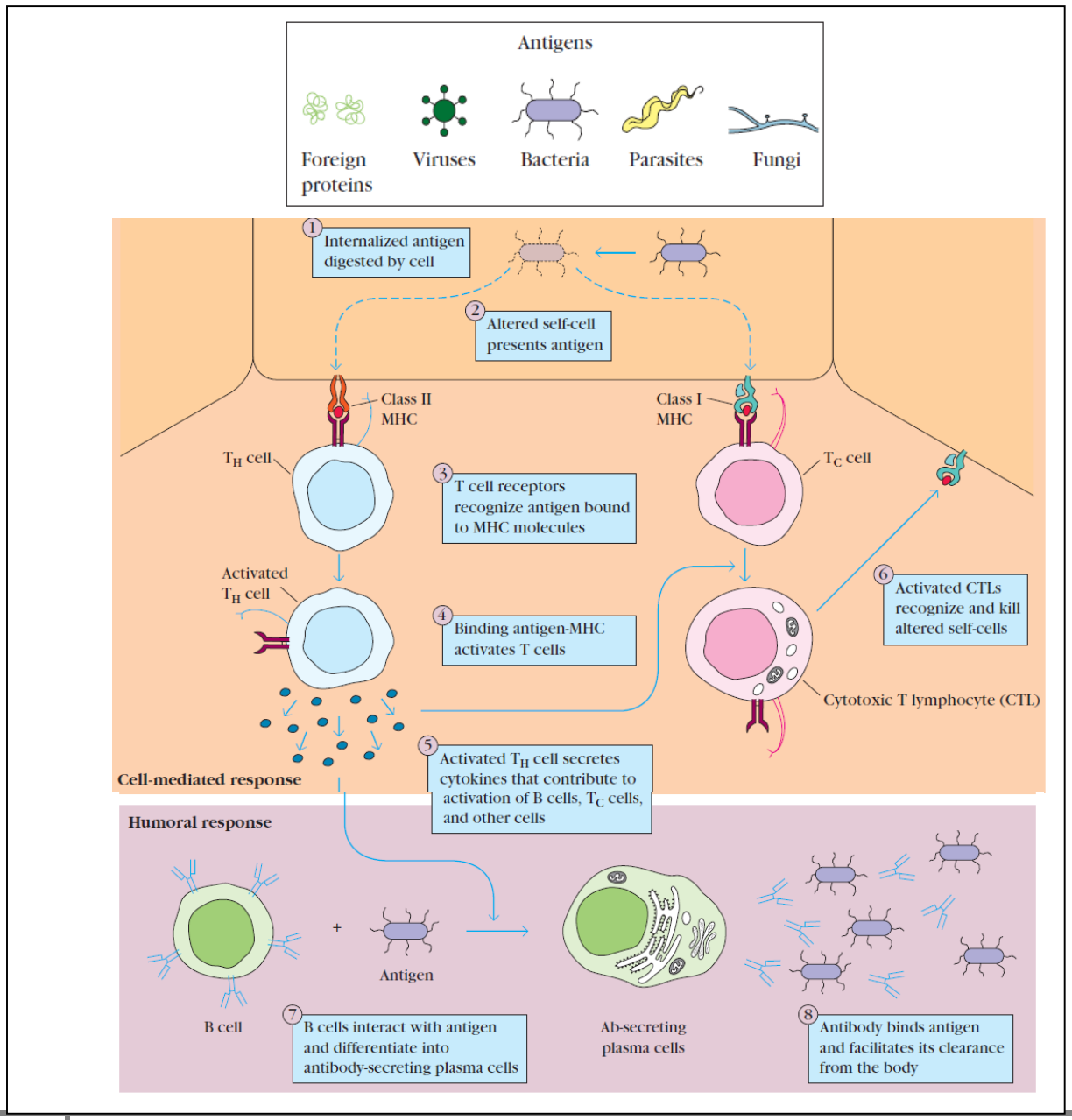
Primary lymphoid organs are those in which the maturation of T and B lymphocytes into antigen recognizing lymphocytes occurs. **Bone marrow** and **thymus** are examples of primary (central) lymphoid organs.

Secondary lymphoid organs are those organs in which antigen driven proliferation and differentiation take place. The mature B and T lymphocytes migrate from the bone marrow and the thymus through the blood stream to the secondary lymphoid organs.

The major secondary lymphoid organs are the **spleen**, the **lymph nodes** and **mucosa associated lymphoid tissue (MALT).** They carry out two main functions: they are highly efficient in trapping and concentrating foreign substances and they are the main sites of production of antibodies and induction of antigen-specific T-lymphocytes.

Humoral and Cell-mediated responses:

- ✓ As mentioned earlier, immune responses can be divided into humoral and cell-mediated responses. Humoral immunity refers to immunity that can be conferred upon a non immune individual by administration of serum antibodies from an immune individual.
- ✓ In contrast, cell-mediated immunity can be transferred only by administration of T cells from an immune individual.
- ✓ The humoral branch of the immune system is at work in the interaction of B cells with antigen and their subsequent proliferation and differentiation into antibody-secreting plasma cells.
- ✓ Antibody functions as the effector of the humoral response by binding to antigen and neutralizing it or facilitating its elimination.
- ✓ When an antigen is coated with antibody, it can be eliminated in several ways. Effector T cells generated in response to antigen are responsible for cell-mediated immunity activated TH cells and cytotoxic T lymphocytes (CTLs) serve as effector cells in cell-mediated immune reactions.
- ✓ Cytokines secreted by TH cells can activate various phagocytic cells, enabling them to phagocytose and kill microorganisms more effectively.



Overview of humoral and cell mediated branches of immune system

Major Histocompatability Complex (MHC)

- ✓ MHC is a tightly linked cluster of genes present in every vertebrate species. MHC is located on chromosome 6 in humans and referred to HLA (human leukocyte antigen) complex.
- ✓ There are two major types of MHC molecules: Class I MHC molecules, which are expressed by nearly all nucleated cells of vertebrate species, consist of a heavy chain linked to a small invariant protein called β2-microglobulin.
- ✓ Class II MHC molecules, which consist of an alpha and a beta glycoprotein chain, are expressed only by antigen-presenting cells. When a naive T cell encounters antigen combined with a MHC molecule on a cell, the T cell proliferates and differentiates into memory T cells and various effector T cells.
- ✓ T_H cells generally recognize antigen combined with class II molecules, whereas T_C cells generally recognize antigen combined with class I molecules.
- ✓ Each MHC molecule can bind to a spectrum of **antigenic peptides** derived from the intracellular degradation of antigen molecules.
- ✓ Class I MHC genes codes for a transmembrane glycoprotein of approximate molecular weight of 43kDa referred to as α or heavy chain. The highest levels of class I molecules are expressed by lymphocytes.
- ✓ Class II MHC genes code for α and β chains of approximate molecular weight 35,000 and 28,000 Da. They are glycoprotein molecules with cytoplasmic tails and extracellular Ig like domains. APC's like fibroblasts, thymic epithelial cells, glial cells can be induced to express class II MHC molecules.

ANTIGENS

- ✓ Adaptive immune responses arise as a result of exposure to foreign compounds. The compound that evokes the response is referred to as antigen against which an antibody is generated.
- ✓ An antigen is any agent capable of binding specifically to T-cell receptor or an antibody molecule. The ability to bind with an Ab or T-cell receptor is referred to as **antigenicity**.
- ✓ An **immunogen** is any agent capable of inducing an immune response and therefore is termed as **immunogenic**.
- ✓ The distinction between the two terms is that there are many compounds that are incapable of inducing an immune response, yet they are capable of binding with components of the immune system that have been induced against them. Thus all immunogens are antigens, but not all antigens are immunogens.

Requirements for immunogenicity:

1. **Foreignness**: An effective immunogen must be foreign with respect to the host. The adaptive immune system recognizes and eliminates only foreign (nonself) antigens.
2. **Size**: The immunogen must have a certain minimal molecular weight. Small compounds with the molecular weight <1000Da (e.g. penicillin, asprin) are not immunogenic, those with the molecular weight between 1000 and 6000 Da (e.g. insulin, adrenocorticotropic hormone) may or may not be immunogenic, and those of molecular weight >6000 Da (e.g. albumin, tetnus toxin) are generally immunogenic.
   The most active immunogens tend to have a molecular mass of 100,000 Da or more. Therefore small substances have decreased immunogenicity and large substances have increased immunogenicity.
3. **Chemical complexity:** The immunogen needs to be chemical complex. Irrespective of the molecular mass of the compounds they must possess a certain level of complexity.

All proteins are immunogenic. The greater the degree of complexity of the protein the more vigorous will be the immune response to that protein. Carbohydrates are immunogenic only if they have a complex polysaccharide structure. Nucleic acids and lipids are poor immunogens but they become immunogenic when they are conjugated to protein carriers.

4. **Dosage and route of administration**: Insufficient dose of the immunogen may not stimulate an immune response because the amount administered would fail to activate enough lymphocytes or the cells may be unresponsive to the dose. Besides the need to administer a certain amount of immunogen to induce an immune response, the number of doses administered also affects the outcome of the immune response.

   The route of administration also plays an important role. Immunogens can be administered through a number of common routes: *intravenous* (into a vein), *intradermal* (into the skin), *subcutaneous* (beneath the skin), *intramuscular* (into the muscle). Antigens administered via the most common route namely, subcutaneous generally elicit the strongest immune responses.

Haptens

Substances called haptens (means to grasp) fail to induce immune responses in their native form because of their low molecular weight and their chemical simplicity. Haptens are antigenic but not immunogenic. These compounds become immunogenic when they conjugated to high molecular weight, physiochemically complex carriers. Thus a hapten by itself is incapable of inducing an immune response; however when it is conjugated to a carrier an immune response is induced.

Antigen-Antibody interactions

Ag-Ab interaction is highly specific and occurs in a similar way like the enzyme substrate complex. The binding between Ag-Ab involves weak non-covalent interactions, Van-der Waals forces, electrostatic forces, hydrophobic forces.

The smallest unit of the antigen that is capable of binding with the antibodies is called as the **antigenic determinant (**epitope**)**. The corresponding are on the antibody molecule combining with the epitope is called **paratope**. The number of epitopes on the surface of the antigen is its **valence**. Valence determines the number of antibody molecules that can combine with the antigen at one time. If one epitope is present the antigen is **monovalent**. More than one copy of the same epitope is called **polyvalent**, seen in most antigens.

Affinity and Avidity

The intrinsic association constant that characterizes the non covalent interaction between antigen binding sites of an antibody (paratope) with an epitope is termed as **affinity**. Low affinity antibodies bind antigen weakly and tend to dissociate readily, where else high affinity antibodies bind antigen more tightly and remain bound longer.

The term **avidity** is used to denote the overall binding between antibodies and a multivalent antigen. So when complex Ag having multiple repeating epitopes is mixed with Ab having multiple binding sites the interaction of such type between multivalent Ag-Ab is called avidity.

Cross reactivity

Although Ag-Ab reaction is very specific sometimes antibody elicited by one antigen can cross react with an unrelated antigen. An immunologic reaction in which a particular antibody or T-cell receptor react with two or more antigens that possess a common epitope is called a cross reaction.

Factors affecting Ag-Ab reaction

The study of Ag-Ab reaction in-vitro is called serology. Serological reactions are the basics for all the diagnostic immunology tests. They depend on a number of factors:

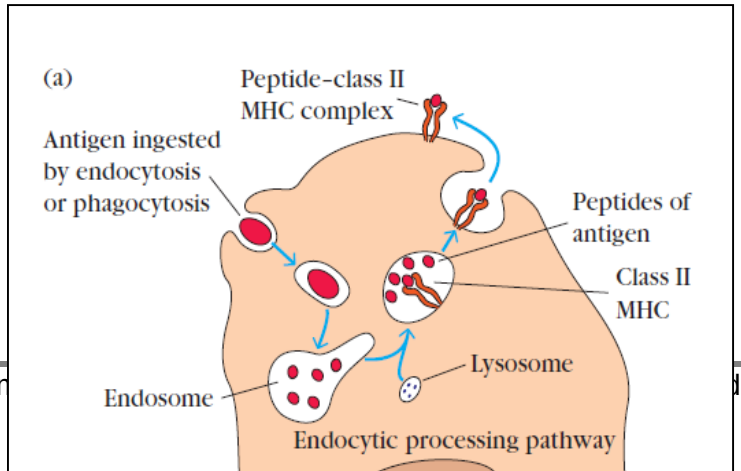
1. Affinity 2. Avidity 3. Antigen: Antibody ratio 4. Physical form of the antigen

Adjuvant

An adjuvant (means to help) is a substance that when mixed with an immunogen and injected with it enhances the immune response against the immunogen. Adjuvants are often used to boost the immune response in case of weak immunogens or when antigens are available in small amounts. Aluminium potassium sulphate (Alum) is a common adjuvant used for human vaccines.

Antigen processing and presentation

In order for a foreign protein antigen to be recognized by a T cell, it must be degraded into small antigenic peptides that form complexes with class I or class II MHC molecules. This conversion of proteins into MHC-associated peptide fragments is called *antigen processing and presentation.*
Whether a particular antigen will be processed and presented together with class I MHC or class II MHC molecules appears to be determined by the route that the antigen takes to enter a cell.
ENDOCYTIC PATHWAY:
Exogenous antigen is produced outside of the host cell and enters the cell by endocytosis or phagocytosis. Antigen presenting cells (macrophages, dendritic cells, and B cells) degrade ingested exogenous antigen into peptide fragments within the endocytic processing pathway.
Class II MHC molecules are expressed within the endocytic processing pathway and those peptides produced by degradation of antigen in this pathway bind to the cleft within the class II MHC molecules. The MHC molecules bearing the peptide are then exported to the cell surface. Since expression of class II MHC molecules is limited to antigen- presenting cells, presentation of exogenous peptide– class II MHC complexes is limited to these cells. T cells displaying $CD4^+$ recognize antigen combined with class II MHC molecules and thus are said to be *class II MHC restricted.* These cells generally function as T helper cells.

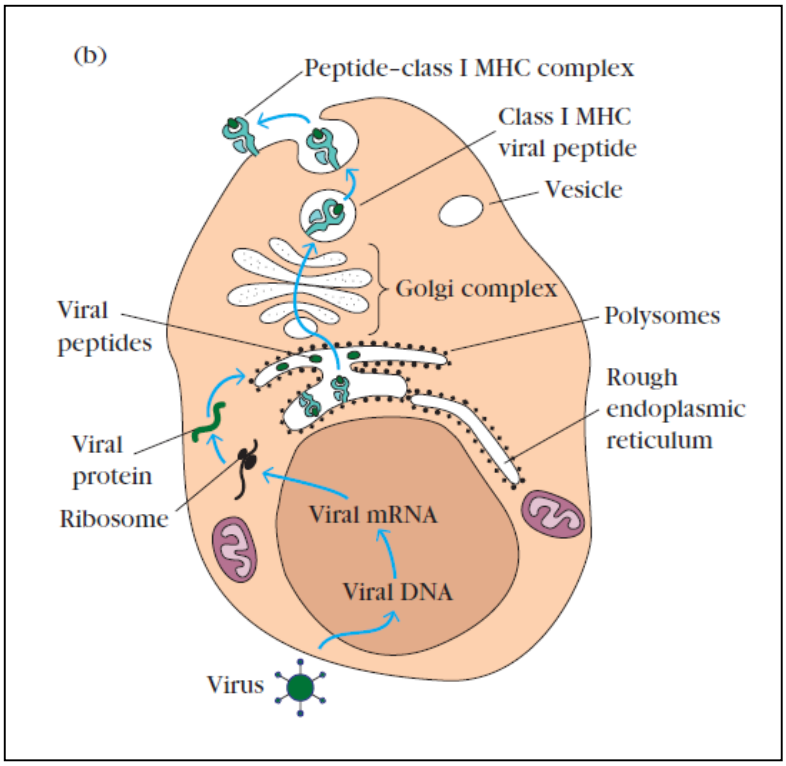


The processing of an exogenous protein antigen for presentation to a helper T-cell.

CYTOSOLIC PATHWAY:

Endogenous antigen is produced within the host cell itself. Two common examples are viral proteins synthesized within virus-infected host cells and unique proteins synthesized by cancerous cells. Endogenous antigens are degraded into peptide fragments that bind to class I MHC molecules within the endoplasmic reticulum. The peptide–class I MHC complex is then transported to the cell membrane. Since all nucleated cells express class I MHC molecules, all cells producing endogenous antigen use this route to process the antigen.

T cells displaying $CD8^+$ recognize antigen associated with class I MHC molecules and thus are said to be *class I MHC restricted.* These cytotoxic T cells attack and kill cells displaying the antigen–MHC class I complexes for which their receptors are specific.



The processing of an endogenous protein antigen for presentation to a cytotoxic T-cell.

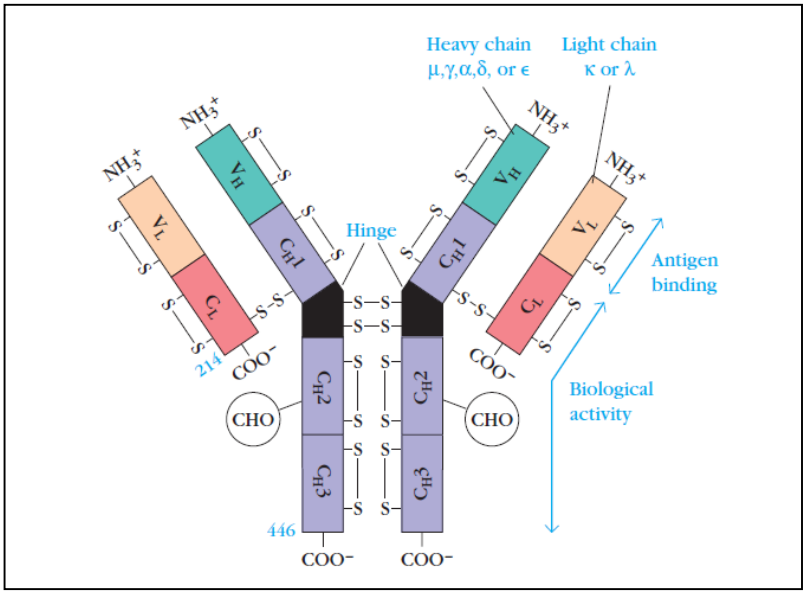
ANTIBODIES AND IMMUNOGLOBULINS

Antibodies

- ✓ **Antibodies are** present on the B-cell membrane and secreted by plasma cells. Membrane-bound antibody confers antigenic specificity on B cells; antigen-specific proliferation of B-cell clones is elicted by the interaction of membrane antibody with antigen.
- ✓ Secreted antibodies circulate in the blood, where they serve as the effectors of humoral immunity by searching out and neutralizing antigens or marking them for elimination.
- ✓ Most antigens are complex and contain many different antigenic determinants, and the immune system usually responds by producing antibodies to several epitopes on the antigen.

Basic structure of antibody

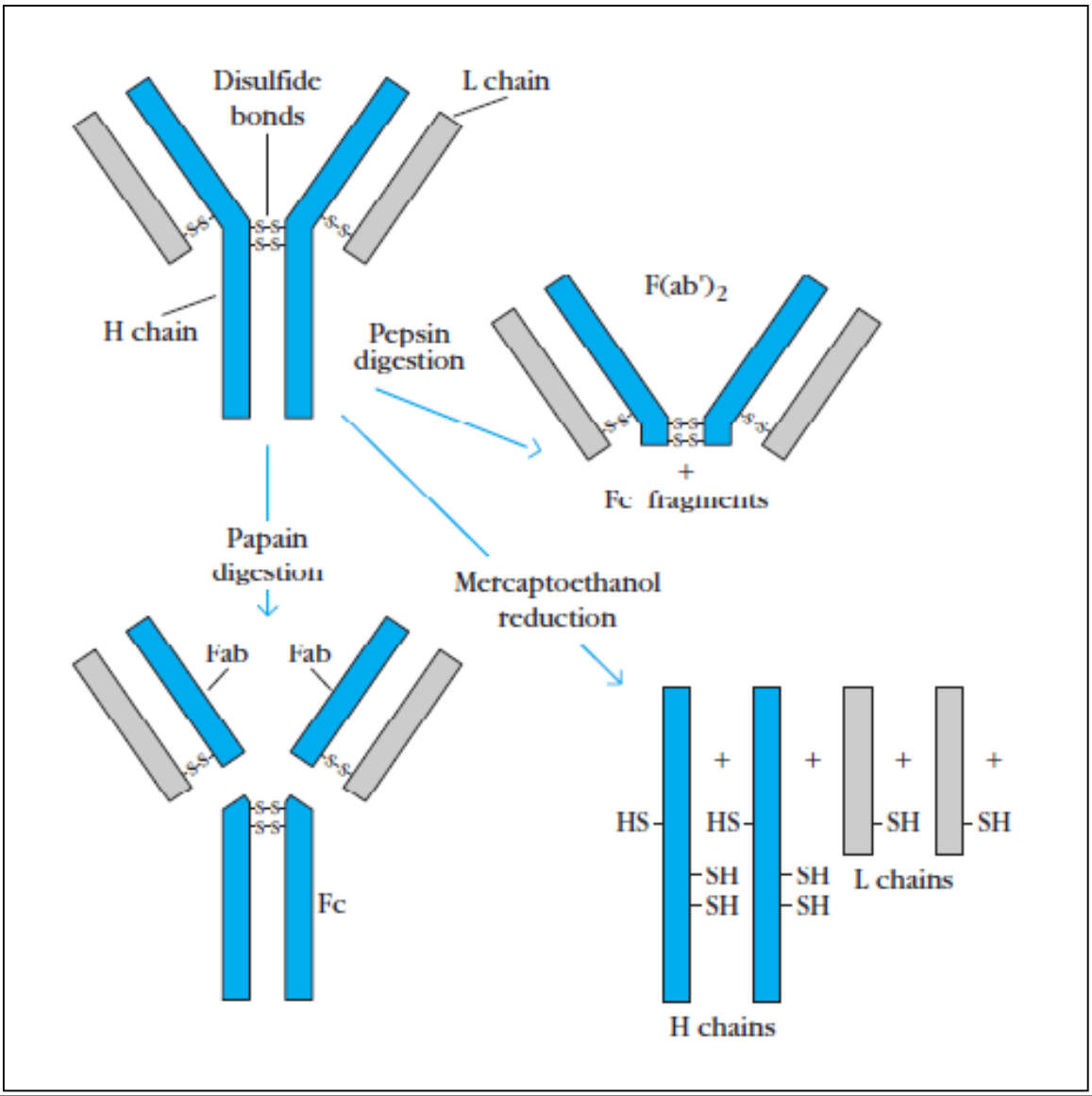
- ✓ Blood when separated in a centrifuge gives out fluid and a cellular fraction. The fluid fraction is the **plasma** and the cellular fraction contains red blood cells, leukocytes, and platelets. Plasma contains all of the soluble small molecules and macromolecules of blood, including fibrin and other proteins required for the formation of blood clots. If the blood or plasma is allowed to clot, the fluid phase that remains is called **serum.**
- ✓ It has been known that antibodies reside in the serum. The first evidence that antibodies were contained in particular serum protein fractions came from a classic experiment by A.Tiselius and E. A.Kabat, in 1939.
- ✓ Antibody molecules have a common structure of four peptide chains. This structure consists of two identical **light (L) chains,** polypeptides of about 25,000 molecular weight, and two identical **heavy (H) chains,** larger polypeptides of molecular weight 50,000 or more.
- ✓ Like the antibody molecules they constitute, H and L chains are also called immunoglobulins. Each light chain is bound to a heavy chain by a disulfide bond, and by such non covalent interactions as salt linkages, hydrogen bonds, and hydrophobic bonds, to form a heterodimer (H-L).
- ✓ The first 110 or so amino acids of the amino-terminal region of a light or heavy chain vary greatly among antibodies of different specificity. These segments of highly variable sequence are called *V regions:* VL in light chains and VH in heavy.
- ✓ Antibodies are glycoproteins; with few exceptions, the sites of attachment for carbohydrates are restricted to the constant region.



Antibody basic structure

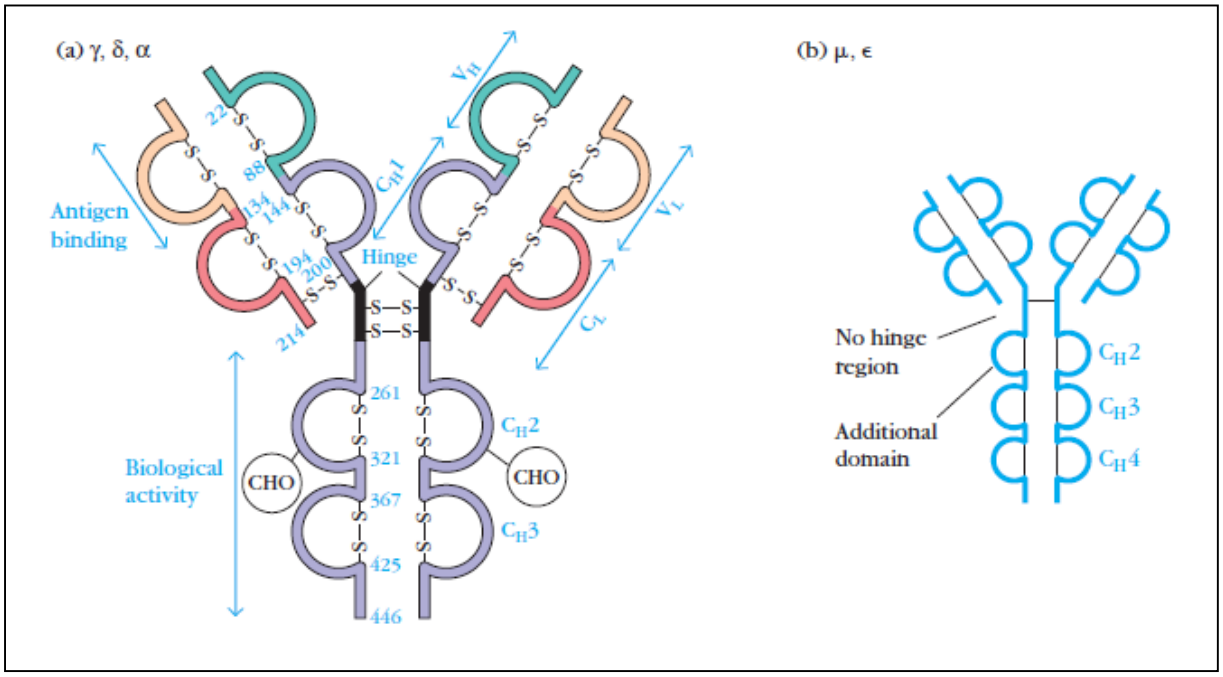
Deduction of antibody structure

- ✓ When the gamma globulin fraction of serum was separated into high and low molecular weight fractions, antibodies of around 150,000-MW, designated as immunoglobulin G (IgG) were found in the low molecular weight fraction.
- ✓ Brief digestion of IgG with the enzyme papain produced three fragments, two of which were identical fragments and a third that was quite different.
- ✓ The two identical fragments (each with a MW of 45,000), had antigen-binding activity and were called **Fab fragments** ("fragment, antigen binding"). The other fragment (MW of 50,000) had no antigen binding activity at all. Because it was found to crystallize during cold storage, it was called the **Fc fragment** ("fragment, crystallizable").
- ✓ Digestion with pepsin, a different proteolytic enzyme, also demonstrated that the antigen-binding properties of an antibody can be separated from the rest of the molecule.
- ✓ Pepsin digestion generated a single 100,000 MW fragment composed of two Fab-like fragments designated the **F (ab')$_2$ fragment,** which binds antigen. The Fc fragment was not recovered from pepsin digestion because it had been digested into multiple fragments.



IMMUNOGLOBULINS

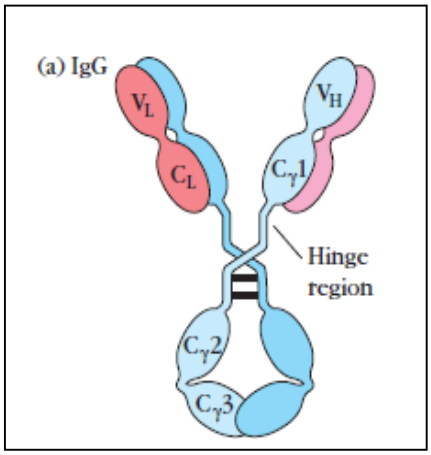
- ✓ For heavy-chain sequencing studies, myeloma proteins were reduced with mercaptoethanol and alkylated, and the heavy chains were separated by gel filtration in a denaturing solvent.
- ✓ The amino-terminal part of the chain, consisting of 100–110 amino acids, showed great sequence variation among myeloma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



- ✓ The γ, δ and α heavy chain contain an extended peptide sequence between the CH_1 and a CH_2 domain that has no homology with the other domains. This region, called the **hinge region,** is rich in proline residues and is flexible, giving IgG, IgD, and IgA segmental flexibility. As a result, the two Fab arms can assume various angles to each other when antigen is bound.

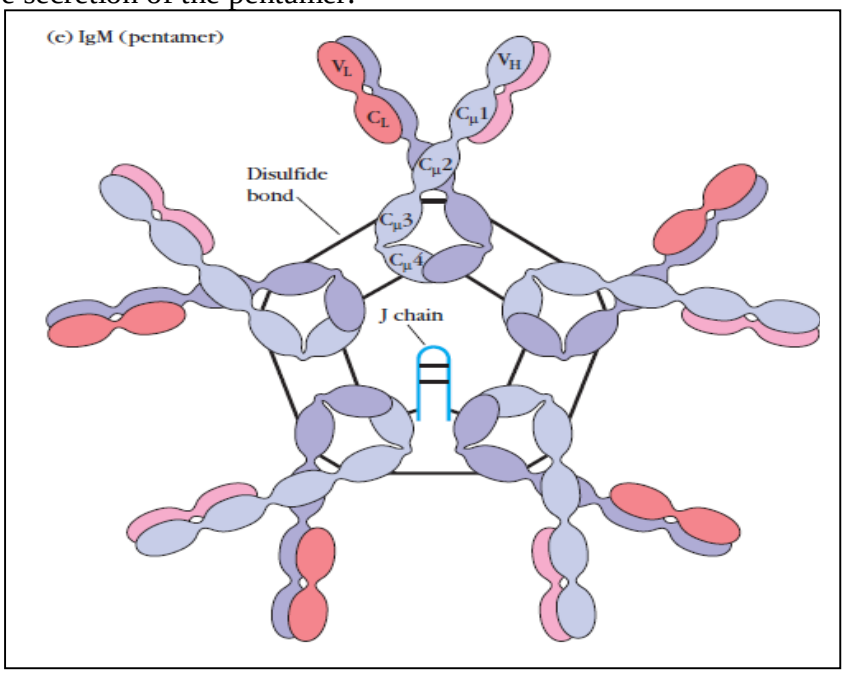
Immunoglobulin G (IgG)

- ✓ IgG, the most abundant class in serum, constitutes about 80% of the total serum immunoglobulin. The IgG molecule consists of two γ heavy chains and two κ or two λ light chains.
- ✓ There are four human IgG subclasses, distinguished by differences in γ chain sequence and numbered according to their decreasing average serum concentrations: IgG1, IgG2, IgG3, and IgG4
- ✓ Amino acid differences between subclasses of IgG affect the biological activity of the molecule: IgG1, IgG3, and IgG4 readily cross the placenta and play an important role in protecting the developing fetus.
- ✓ IgG3 is the most effective complement activator, followed by IgG1; IgG2 is less efficient, and IgG4 is not able to activate complement at all.
- ✓ IgG1 and IgG3 bind with high affinity to Fc receptors on phagocytic cells and thus mediate opsonization. IgG4 has an intermediate affinity for Fc receptors, and IgG2 has an extremely low affinity.



Immunoglobulin M (IgM)

- ✓ IgM accounts for 5%–10% of the total serum immunoglobulin, with an average serum concentration of 1.5 mg/ml. Monomeric IgM, with a molecular weight of 180,000, is expressed as membrane-bound antibody on B cells. IgM is secreted by plasma cells as a pentamer in which five monomer units are held together by disulfide bonds.
- ✓ The five monomer subunits are arranged with their Fc regions in the center of the pentamer and the ten antigen-binding sites on the periphery of the molecule. Each pentamer contains an additional Fc-linked polypeptide called the **J (joining) chain,** which is disulfide-bonded to the carboxyl-terminal cysteine residue of two of the ten μ chains.
- ✓ The J chain appears to be required for polymerization of the monomers to form pentameric IgM; it is added just before secretion of the pentamer.

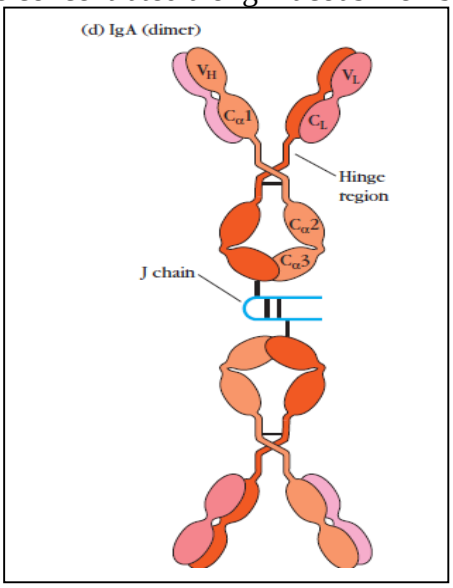


- ✓ IgM is the first immunoglobulin class produced in a primary response to an antigen, and it is also the first immunoglobulin to be synthesized by the neonate. Because of its pentameric structure with 10 antigen-binding sites, serum IgM has a higher valency than the other isotypes.
- ✓ An IgM molecule can bind 10 small hapten molecules; however, because of steric hindrance, only 5 or fewer molecules of larger antigens can be bound simultaneously.

- ✓ Because of its high valency, pentameric IgM is more efficient than other isotypes in binding antigens with many repeating epitopes such as viral particles and red blood cells (RBCs).
- ✓ Because of its large size, IgM does not diffuse well and therefore is found in very low concentrations in the intercellular tissue fluids. The presence of the J chain allows IgM to bind to receptors on secretory cells, which transport it across epithelial linings to enter the external secretions that bathe mucosal surfaces.
- ✓ Although IgA is the major isotype found in these secretions, IgM plays an important accessory role as a secretory immunoglobulin.

Immunoglobulin A (IgA)

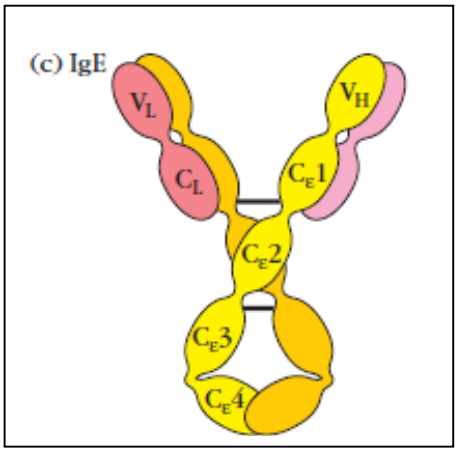
- ✓ Although IgA constitutes only 10%–15% of the total immunoglobulin in serum, it is the predominant immunoglobulin class in external secretions such as breast milk, saliva, tears, and mucus of the bronchial, genitourinary, and digestive tracts.
- ✓ In serum, IgA exists primarily as a monomer, but polymeric forms (dimers, trimers, and some tetramers) are sometimes seen, all containing a J-chain polypeptide. The IgA of external secretions, called **secretory IgA,** consists of a dimer or tetramer, a J-chain polypeptide, and a polypeptide chain called **secretory component**.
- ✓ The secretory component is derived from the receptor that is responsible for transporting polymeric IgA across cell membranes. The J-chain polypeptide in IgA is identical to that found in pentameric IgM and serves a similar function in facilitating the polymerization of both serum IgA and secretory IgA.
- ✓ The secretory component is a 70,000-MW polypeptide produced by epithelial cells of mucous membranes. It consists of five immunoglobulin-like domains that bind to the Fc region domains of the IgA dimer.
- ✓ This interaction is stabilized by a disulfide bond between the fifth domain of the secretory component and one of the chains of the dimeric IgA. The daily production of secretory IgA is greater than that of any other immunoglobulin class.
- ✓ IgA-secreting plasma cells are concentrated along mucous membrane surfaces.



Immunoglobulin E (IgE)

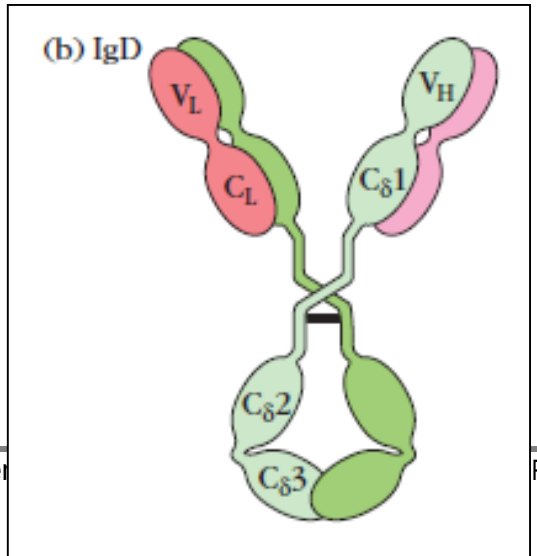
- ✓ The potent biological activity of IgE allowed it to be identified in serum despite its extremely low average serum concentration (0.3µg/ml).
- ✓ IgE antibodies mediate the immediate hypersensitivity reactions that are responsible for the symptoms of hay fever, asthma, hives, and anaphylactic shock.

- ✓ The presence of a serum component responsible for allergic reactions was first demonstrated in 1921 by K. Prausnitz and H. Kustner, who injected serum from an allergic person intra-dermally into a non allergic individual. When the appropriate antigen was later injected at the same site, a wheal and flare reaction (analogous to hives) developed there. This reaction, called the **P-K reaction** (named for its originators, Prausnitz and Kustner), was the basis for the first biological assay for IgE activity.
- ✓ IgE binds to Fc receptors on the membranes of blood basophils and tissue mast cells. Cross-linkage of receptor bound IgE molecules by antigen (allergen) induces basophils and mast cells to translocate their granules to the plasma membrane and release their contents to the extracellular environment, a process known as degranulation.
- ✓ As a result, a variety of pharmacologically active mediators are released and give rise to allergic manifestations



Immunoglobulin D (IgD)

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



ANTIGENIC DETERTMINANTS

- ✓ The antigenic determinants, or epitopes, on immunoglobulin molecules fall into three major categories: isotypic, allotypic, and idiotypic determinants, which are located in characteristic portions of the molecule.

ISOTYPE

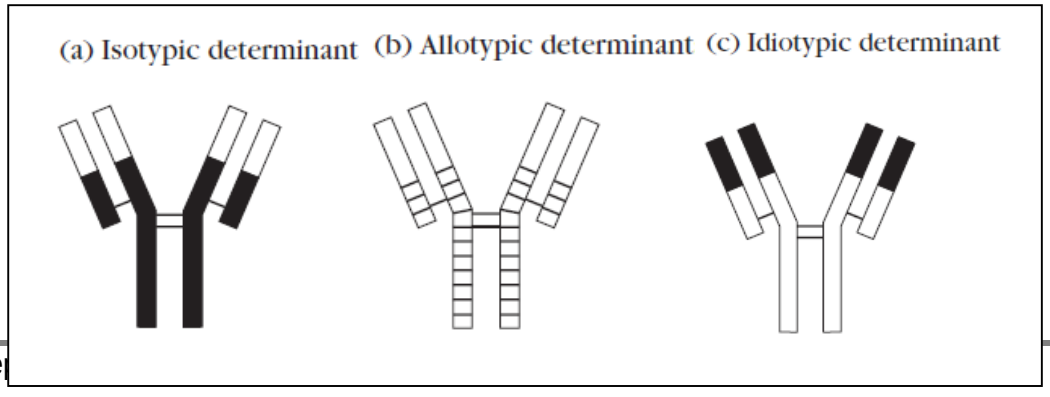
- ✓ Isotypic determinants are constant-region determinants that collectively define each heavy-chain class and subclass and each light-chain type and subtype within a species.
- ✓ Each isotype is encoded by a separate constant region gene, and all members of a species carry the same constant-region genes (which may include multiple alleles).
  Therefore, when an antibody from one species is injected into another species, the isotypic determinants will be recognized as foreign, inducing an antibody response to the isotypic determinants on the foreign antibody.

ALLOTYPE

- ✓ Although all members of a species inherit the same set of isotype genes, multiple alleles exist for some of the genes. These alleles encode subtle amino acid differences, called allotypic determinants that occur in some, but not all, members of a species. The sum of the individual allotypic determinants displayed by an antibody determines its **allotype.**
- ✓ Antibody to allotypic determinants can be produced by injecting antibodies from one member of a species into another member of the same species who carries different allotypic determinants. Antibody to allotypic determinants sometimes is produced by a mother during pregnancy in response to paternal allotypic determinants on the fetal immunoglobulins. Antibodies to allotypic determinants can also arise from a blood transfusion.

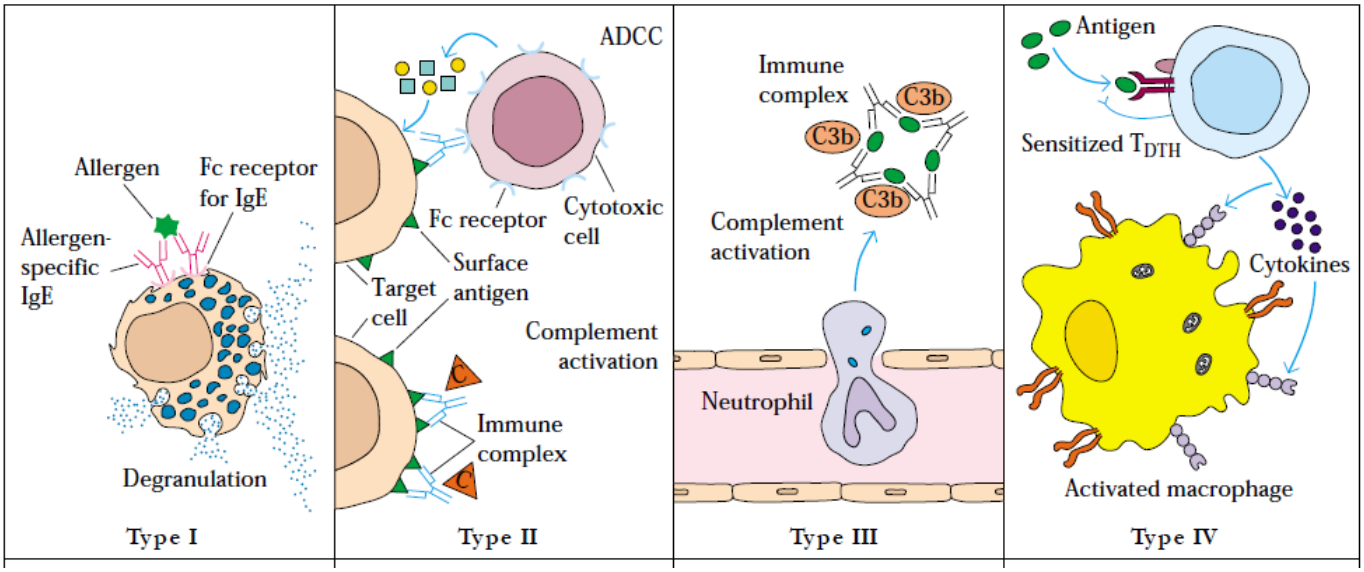
IDIOTYPE

- ✓ The idiotypic determinants arise from the sequence of the heavy- and light-chain variable regions. Each individual antigenic determinant of the variable region is referred to as an **idiotope**
- ✓ In some cases an idiotope may be the actual antigen-binding site, and in some cases an idiotope may comprise variable-region sequences outside of the antigen binding site. Each antibody will present multiple idiotopes;the sum of the individual idiotopes is called the **idiotype** of the antibody.
- ✓ Often a homogeneous antibody such as myeloma protein or monoclonal antibody is used. Injection of such an antibody into a recipient who is genetically identical to the donor will result in the formation of anti-idiotype antibody to the idiotypic determinants.



HYPERSENSITIVITY

- ✓ An immune response mobilizes a battery of effector molecules that act to remove antigen by various mechanisms. Generally these effector molecules induce a localized inflammatory response that eliminates antigen without extensively damaging the host's tissue.
- ✓ Under certain circumstances, however, this inflammatory response can have deleterious effects, resulting in significant tissue damage or even death. This inappropriate immune response is termed **hypersensitivity** or **allergy.**
- ✓ Anaphylactic reactions within the humoral branch initiated by antibody or antigen-antibody complexes is **called immediate hypersensitivity,** because the symptoms are manifest within minutes or hours after a sensitized recipient encounters antigen.
- ✓ **Delayed-type hypersensitivity (DTH)** is so named in recognition of the delay of symptoms until days after exposure.



IgE-Mediated Hypersensitivity	IgG-Mediated Cytotoxic Hypersensitivity	Immune Complex-Mediated Hypersensitivity	Cell-Mediated Hypersensitivity
Ag induces crosslinking of IgE bound to mast cells and basophils with release of vasoactive mediators	Ab directed against cell surface antigens meditates cell destruction via complement activation or ADCC	Ag-Ab complexes deposited in various tissues induce complement activation and an ensuing inflammatory response mediated by massive infiltration of neutrophils	Sensitized T_H1 cells release cytokines that activate macrophages or T_C cells which mediate direct cellular damage
Typical manifestations include systemic anaphylaxis and localized anaphylaxis such as hay fever, asthma, hives, food allergies, and eczema	Typical manifestations include blood transfusion reactions, erythroblastosis fetalis, and autoimmune hemolytic anemia	Typical manifestations include localized Arthus reaction and generalized reactions such as serum sickness, necrotizing vasculitis, glomerulnephritis, rheumatoid arthritis, and systemic lupus erythematosus	Typical manifestations include contact dermatitis, tubercular lesions and graft rejection

The four types of hypersensitive responses.

SEROLOGY

- ✓ Serology is the scientific study of serum and other bodily fluids. In practice the term usually refers to the diagnostic identification of antibodies in the serum. Such antibodies are typically formed in response to an infection (against a given microorganism), against other foreign proteins (mismatched blood transfusion) or to one's own proteins (autoimmune diseases).
- ✓ Serological tests may be performed for diagnostic purpose when an infection is suspected, and in many other situations such as checking individual's blood type. Serological blood tests help to diagnose patients with certain immune deficiencies associated with the lack of antibodies.
- ✓ There are several serological techniques that can be used depending on the antibodies being studied. These include; ELISA, agglutination, precipitation, complement fixation and fluorescent antibodies. Some serological tests are not limited to blood serum, but can also be performed on other body fluids such as semen and saliva, which have (roughly) similar properties to serum. Serological tests may also be used in forensic serology, specifically for a piece of evidence.