1. Basic Components of the Immune System

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INTRODUCTION

This chapter is not a comprehensive review of immunology but rather a condensed version of those aspects of immunology that have particular relevance to clinical immunology. Refer to the Bibliography for a more extensive discussion of the role of each component.

It is generally believed that the immune system evolved as the host's defense against infectious agents, and it is well known that patients with deficiencies in the immune system generally succumb to these infectious diseases. However, as we shall see, it may well play a larger role in the elimination of other foreign substances, including tumor antigens or cells and antibodies that attack self.

An immune response may be conveniently divided into two parts: (1) a specific response to a given antigen and (2) a more nonspecific augmentation to that response. An important feature of the specific response is that there is a quicker response to the antigen during a second exposure to that antigen. It is the memory of the initial response that provides the booster effect.

For convenience, the specific immune response may be divided into two parts: (1) the humoral response and (2) the cellular response to a given antigen. As we shall see, however, both responses are mediated through the lymphocyte. Humoral responses are antibodies produced in response to a given antigen, and these antibodies are proteins, have similar structures, and can be divided into various classes of immunoglobulins. Cellular responses are established by cells and can only be transferred by cells. (See the Bibliography for the extraordinary beginnings of the concept of a cellular arm of the immune system.) Up to the 1940s the general dogma held that only antibodies were involved in the immune response. Dr. Merrill Chase, who began his experiments in a laboratory devoted primarily to the humoral response, clearly showed in a series of elegant experiments that immunity was not just humoral but that a cellular response by the lymphocytes could also produce immunity. Some of the best examples of the power of cellular immunity may be found in the many experiments in which transfer of cells can induce autoimmune disease in animals and humans as well as rejection of an organ graft in both animals and humans by cells.

The separation of human and cellular immunity was further advanced by the study of immunodeficient humans and animals. For example, thymectomized or congenitally athymic animals as well as humans cannot carry out graft rejection, yet they are capable of producing some antibody responses. The reverse is also true. Children (and animals) who have an immune deficit in the humoral response do not make antibodies but can reject

grafts and appear to handle viral, fungal, and some bacterial infections quite well. An extraordinary finding by Good and colleagues in studying the cloacal lymphoid organ in chickens revealed that, with removal of the bursa Fabricius, these animals lost their ability to produce antibodies and yet retained the ability to reject grafts.

Out of these and many other contributions, a clearer picture of the division of efforts by lymphocytes begins to emerge. Since cellular immune responses require an intact thymus, cellular immune responses are mediated through the T lymphocytes (thymus), while antibody-producing cells, which are dependent on the bone marrow (the bursa equivalent), are known as B (bursa) cells. The pathways of both cell types are depicted in Figure 1.1.

Several types of molecules play a vital role in the immune response, and we will deal with each in detail. Antigens, both foreign and self, are substances that may or may not provoke an immune response. Both T cells and B cells have receptors that recognize these antigens. In the case of B cells, antibodies on the surface are a major source (but not the only one) of antigen recognition, and once activated, they differentiate into plasma cells that produce large quantities of antibodies that are secreted into blood and body fluids to block the harmful effects of the antigen.

T cells have similar receptors known as T-cell receptors (TCR), and in the context of the major histocompatibility complex (MHC) molecules provide a means of self-recognition and T-lymphocyte effector functions. Often these effector functions

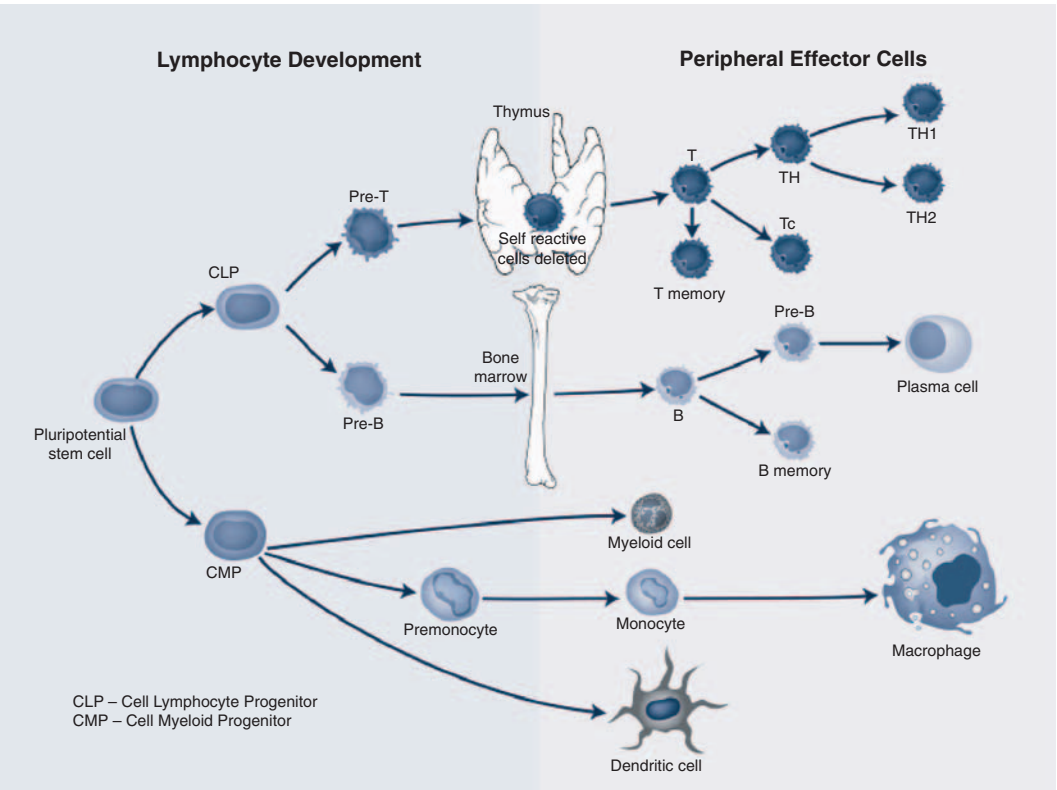

Figure 1.1 Development and differentiation of lymphocytes from pluripotential stem cells.

are carried out by messages transmitted between these cells. These soluble messengers are called *interleukins* or *cytokines*.

ANTIGENS

Antigens are any substances that are capable, under appropriate conditions, of inducing the formation of antibodies and reacting specifically with the antibodies so produced. They react with both T-cell recognition receptors and with antibodies. These antigenic molecules may have several antigenic determinants, called *epitopes*, and each epitope can bind with a specific antibody. Thus, a single antigen can bind to many different antibodies with different binding sites.

Some low-molecular-weight molecules called *haptens* are unable to evoke an immune response but can react with existing antibodies. These molecules need to be coupled to a carrier molecule to be antigenic.

For some molecules such as drugs, the molecule needs to be conjugated to a carrier. The carrier may be a host protein. The tertiary structure of the molecule as well as the amino acid sequence is important in determining antigenicity. Certain structures such as lipids and DNA are generally poor antigens.

Most antigens are either thymus-dependent or thymus-independent antigens. Thymus-dependent antigens require T-cell participation: Most proteins and foreign red cells are examples of these molecules. Thymus-independent antigens do *not* require T-cell participation for antibody production. Instead, they directly stimulate specific B lymphocytes by cross-linking antigen receptors on the surface of B cells. These molecules produce primarily IgM and IgG_2 antibodies and do not stimulate long-lasting memory cells. Most bacterial polysaccharides (found in bacterial cell walls) fall into this category. Certain polysaccharides, such as LPS (lipopolysaccharide), not only induce specific B-cell activation but also can act as a polyclonal B-cell stimulant.

ANTIBODY

The basic structure of the antibody molecule is depicted in Figures 1.2A and B. It consists of a four-chain structure divided into two identical heavy (H) chains with a molecular weight of 25 kDa. Each chain is composed of *domains* of 110 amino acids and is connected in a loop by a disulfide bond between two cysteine residues in the chain.

The amino acid N-terminal domains of the heavy and light chains include the antigen-binding site. The amino acids of these variable domains vary between different antibody molecules and are thus known as the *variable* (V) regions. Most of these differences reside in the *hypervariable* areas of the molecule and are usually only six to ten amino acid residues in length. When the hypervariable regions in each chain come together along with the counterparts on the other pair of H and L chains, they form the antigen-binding site. This part of the molecule is unique to the molecule and is known as the *idiotype determinant*. In any individual, 10^6 to 10^7 different antibody molecules can be composed from 10^3 different heavy and light chains of the variable regions. The part of the molecule next to the V region is called the constant (C) region made up of one domain in the light chain (C_1) and three or four in a heavy chain (C_H). A C_l chain may consist of either

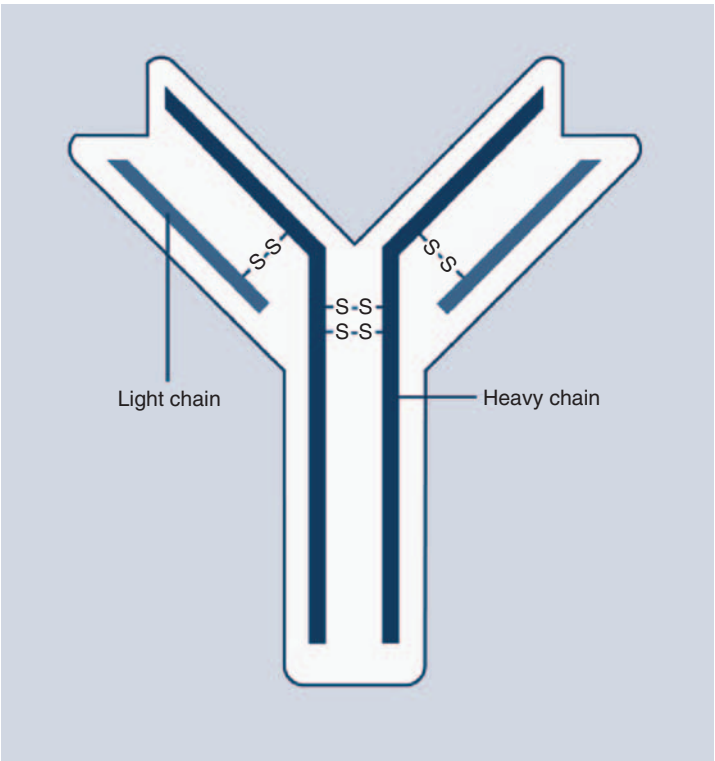

Figure 1.2A Heavy and light chains of an IgG antibody. An IgM antibody would be a pentameric structure of an IgG molecule.

two kappa (κ) or two lambda (λ) chains but *never* one of each. Of all the human antibody molecules, approximately 60%, are κ chains and 40% contain λ chains. Although there are no known differences in the functional properties of κ and λ chains, there are several different types of the C_H domain. These differences are reflected in determining the class (isotype) of the antibody and thereby the physiological function of a particular antibody molecule.

The IgM molecule is the oldest class of immunoglobulins, and it is a large molecule consisting of five basic units held together by a J chain. The major role IgM plays is the intravascular neutralization of organisms, especially viruses. The reason for this important physiological role is that it contains five complement-binding sites, resulting in excellent complement activation. This activation permits the segment removal of antigen–antibody complement complexes via complement receptors on phagocytic cells or complement-mediated lysis of the organism. However, in contrast to the IgG molecule, it has relatively low affinity binding to the antigen in question. Second, because of its size, it does not usually penetrate into tissues.

In contrast, IgG is a smaller molecule that penetrates easily into tissues. There are four major classes of IgG: IgG_1 and IgG_3 activate complement efficiently and clear most protein antigens, including the removal of microorganisms by phagocytic cells. In contrast, IgG_2 and IgG_4 react mostly with carbohydrate antigens and are relatively poor opsonins. This is the only molecule that crosses the placenta to provide immune protection to the neonate.

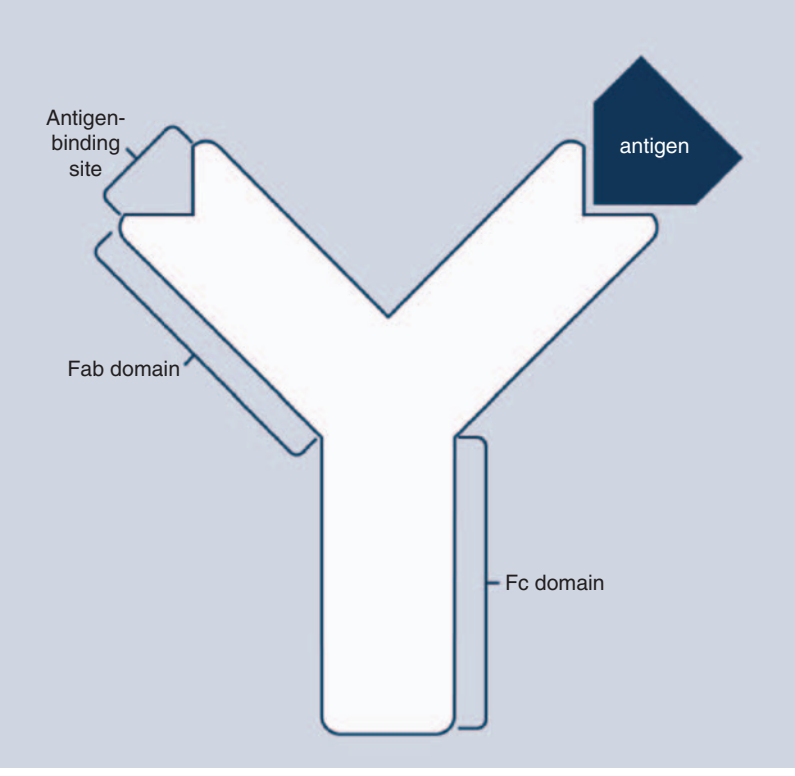

Figure 1.2B Antigen-binding sites and antigen binding in an IgG antibody. Hinge region allows for rotational and lateral movements of the two antigen-binding sites.

The major mucosal immunoglobulin, IgA, consists of two basic units joined by a J chain. The addition of a secretion molecule prevents its digestion by enzymes present in mucosal and intestinal secretions. Thus, IgA_2 is the major IgA molecule in secretions and is quite effective in neutralizing antigens that enter via these mucosal routes. IgA_1, the main IgA molecule in serum, is, however, susceptible to inactivation by serum proteases and is thus less active for defense. Its function is unclear at present.

Two other classes are worthy of note. IgD is synthesized by antigen-sensitive B cells and is involved in the activation of these cells by antigen. IgE is produced by plasma cells and binds to specific IgE receptors on most cells and basophiles. This molecule (see Chapter 9) plays an extremely important role in allergic reactions and expelling intestinal parasites, which is accomplished by increasing vascular permeability and inducing chemotactive factors following mast cell degranulation.

Given this extraordinary ability to generate large numbers of antibody molecules, how does the immune system recognize all pathogens, including past, present, and future? This diversity is achieved by the way in which the genetics of antibody production is arranged (see Figure 1.3). The light and heavy chains are carried on different chromosomes. The heavy chain genes are carried on chromosome 14. These genes are broken up into coding systems called *exons* with intervening segments of silent segments called *entrons*. The exons represent the central region of the heavy

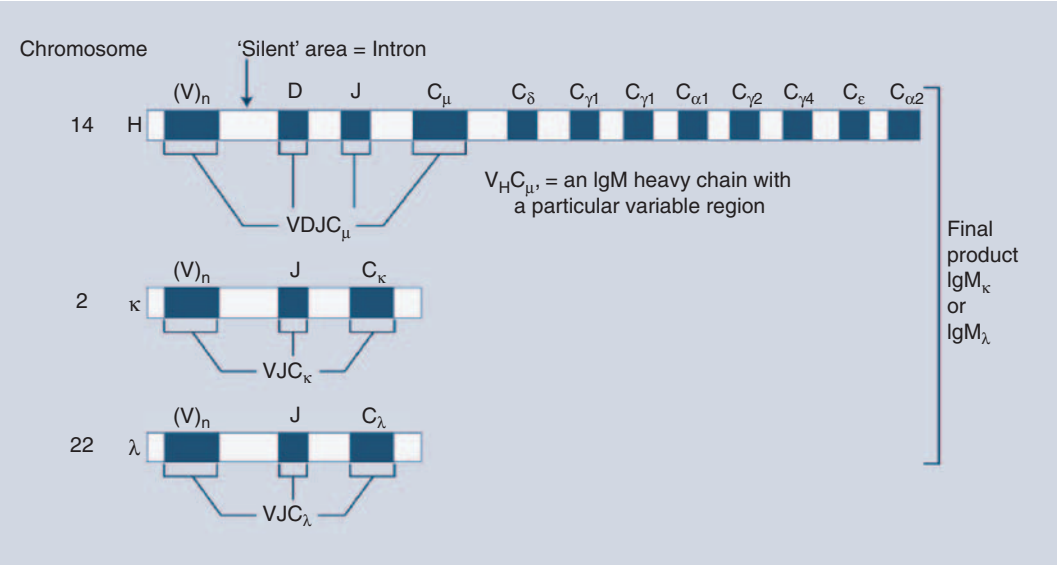

Figure 1.3 The genetics of antibody production.

chain and a large number of V regions. Between the V and D genes are two small sets of exons called the D and J. With each single B cell, one V gene is joined to one D and J in the chromosome. The product, the V_H domain, is then joined at the level of RNA processing to C_u and the B cell makes an IgM molecule. By omitting the C_u gene and joining V_HD_J to a C_λ an IgG molecule is produced. This enormous versatility allows the cell to make IgM, IgD, IgG, IgA, or IgE in sequence while using the same variable regions (see Figure 1.4). The heavy chain gene recombinations are controlled by two recombination activity genes called RAG_1 and RAG_2. If these genes are eliminated by "knock-out" techniques in mice, profound immunodeficiency status occurs in these animals, characterized by absent mature B and T cells.

Thus, the diversity of antigen binding is achieved by the large number of V

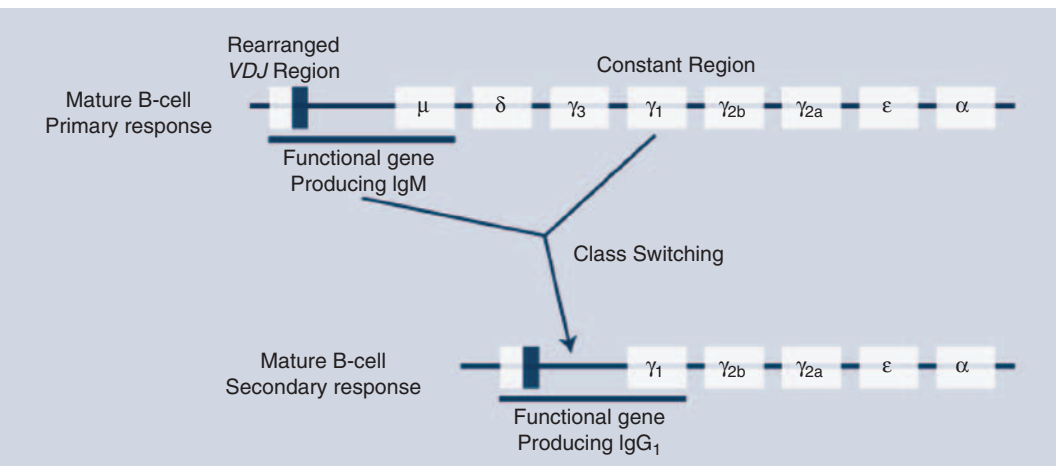

Figure 1.4 Recombination events necessary for generation of class and subclass switching.

genes available and their combination with different D and L genes to provide different antibodies. Furthermore, the inherited set of genes may be increased by somatic mutation during multiple divisions of lymphoid cells, thereby increasing the number of antibody specificities to 10^{14}, which far exceeds the number of B cells (10^{10}) in the body.

Once a given B cell is preselected to produce a particular V_H and V_L domain, all the ensuing progeny of that B cell will produce the same V_H or V_L domain. The sequence of events is as follows: initially, the B cell produces intracellular antigen-specific IgM, which becomes bound to the cell surface. The B cell is now antigen responsive with exposure to a given antigen. The committed B cell begins producing a certain isotype or class of immunoglobulins and begins dividing, and all the progeny will produce the identical immunoglobulin molecules. These B cells will later mature into either plasma cells or long-term memory B cells.

T CELLS AND THEIR RECEPTORS

Each T cell is also committed to a given antigen and recognizes it by one of two TCRs. They may have TCR2s composed of gamma (γ) and delta (δ) chains or TCR2s composed of another heterodimer of alpha (α) and beta (β) chains. These TCR2s are associated with a group of transmembrance proteins on the CD3 molecule, which takes the antigen recognition signal inside the cell. Signal transduction via the CD3 complex is regulated by a series of kinases, which are associated with the tails of the CD3–TCR complex and regulate phosphorylation. Deficiencies or blocks in the T-cell signaling pathways either at the cell-surface complex or at the level of the kinases may result in various forms of immunodeficiency. Two other important antigens present on TCR2 cells recognize histocompatibility antigens and will be discussed later. The genes for TCR chains are on different chromosomes with the β and α molecules on chromosome 7, while the α and δ are on chromosome 14. As seen in Figure 1.5, the four chains are made up of a variable region and a constant region similar to those observed with the immunoglobulins. The variable regions are also numerous and joined at D and J regions by RAG_1 and RAG_2. This permits a diversity of antigen recognition similar to that observed with immunoglobulin, but additional somatic mutation is not involved in T cells. These similarities have led to the concept that genes for antigen-specific T cells evolved in the same manner as immunoglobulin from a parent gene, and both are members of a superantigen family.

The TCR complex recognizes small peptides presented to it by the MHC class I and II and depends on the type of T cell.

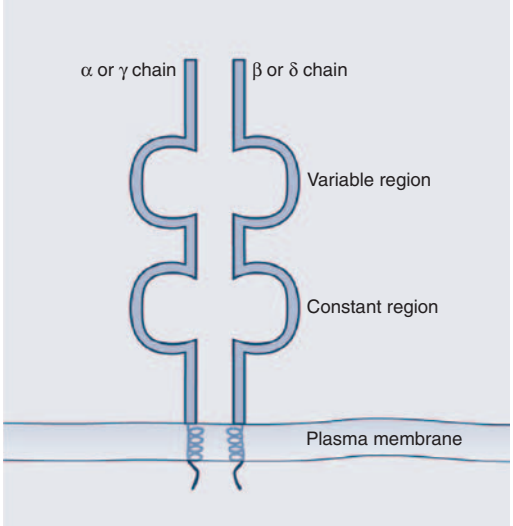

Figure 1.5 Diagram of the structure of a T-cell receptor.

Helper T cells (CD4) recognize class II antigens while suppressor cytotoxic T cells (CD8) recognize class I antigens. Because of the rather low affinity of the reactions, recognition of processed antigen alone is not sufficient to activate T cells. Soluble interleukins are needed to complete the picture and are generated during the antigen processing.

MAJOR HISTOCOMPATIBILITY COMPLEX

Human histocompatibility antigens are also known as human leucocyte antigens (HLA), a term that is synonymous with the MHC complex. These antigens are cell-surface glycoproteins classified as type I or type II. They can produce genetic polymorphism with multiple alleles at each site, thus permitting a great deal of genetic variability between given individuals (see Figure 1.6). This extensive polymorphism is important when viewed in the context of an immune system that needs to cope with an ever-increasing range of pathogens. These pathogens in turn are extremely adept at evading the immune system. Thus, the battle between invading microbe and immune recognition is constant and ever changing. Recognition of antigen by T cells is MHC restricted. Therefore, any given individual is only able to recognize antigen as part of a complex of antigenic peptide and self.

The importance of this concept is underscored by the experiments of Dougherty and Zinkernagel. Using virus-specific (virus 1) cytotoxic T cells, Figure 1.7 illustrates their remarkable discovery. If antigen-presenting cells (APCs) of mouse A are mixed with T cells of mouse A in the context of virus 1 peptides, the T cell responds and kills the virus. If the MHC complex is from mouse B and the T cells

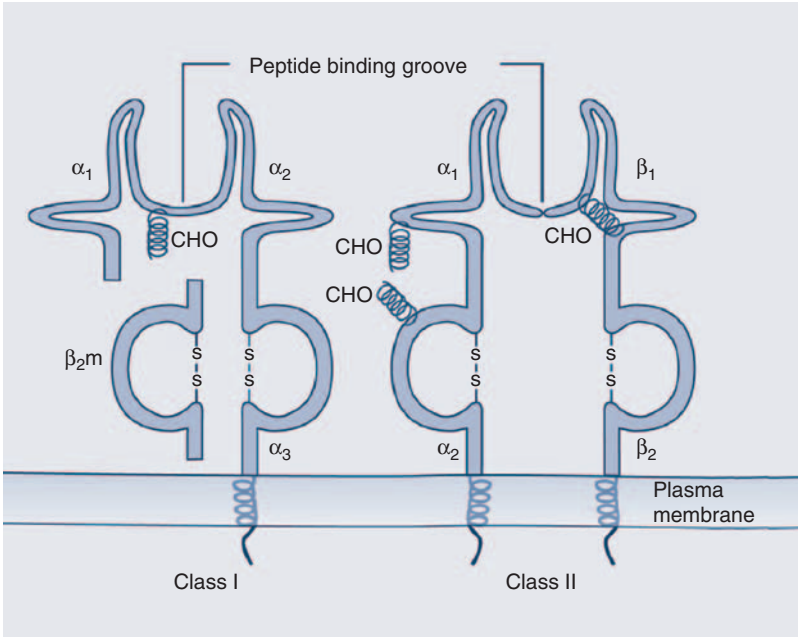

Figure 1.6 Diagrammatic representation of class I and II MHC antigens with B_2 microglobulins and CHO carbohydrate side chains.

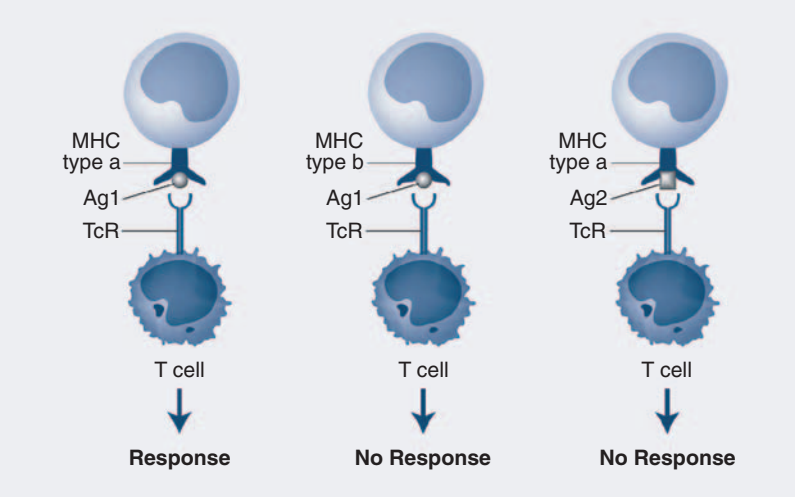

Figure 1.7 MHC restriction of antigen recognition by T cells. If APC and T cell are of the same genetic lineage as virus I, the T cell responds and kills the virus. If APC and T cell are of different lineage, no response occurs. If APC and T cell are of same lineage but virus 2 is present, no response occurs.

are from mouse A, no killing occurs. Finally, if MHC and T cells are both from mouse A but virus 2 is unrelated to virus 1, no response will occur.

The MHC class I antigens are divided into three groups (A, B, and C), and each group belongs on a different gene locus on chromosome 6. The products of all three loci are similar and are made up of a heavy chain (45 kDa) and associated β_2 microglobulin molecule (12 kDa) gene, which resides on chromosome 12. The MHC class I antigen differences are due to variations in the α chains, the β_2 microglobulin being constant. X-ray crystallography studies have shown that as few as nine amino acids can be tightly bound in the α chain groove.

MHC class II antigens also exhibit a similar structure with the groove being formed by the α_1 and β_1 chains. Unlike class I antigens present on most nucleated cells, the class II antigens are restricted to a few types: macrophages, B cells, and activated T cells. In humans, there are three groups of class II antigens: namely, HLA-DP, HLA-DQ, and HLA-DR.

Depending on the nature of the antigen (endogenous or exogenous), the MHC response is different. For example, endogenous antigens (including viral antigens) are presented by MHC class I antigen cells exclusively to CD8 cells. The endogenous antigen is first broken down into small peptides and transported by shuttle proteins called Tap I and Tap II to the endoplasmic reticulum. There they complex with MHC class I molecules and are delivered to the cell surface for further processing to the CD8 cells.

In contrast, MHC class II molecules are held in the endoplasmic reticulum and are protected from binding to peptides in the lumen (not human) by a protein called MHC class II associated invariant chain.

Finally, there are class III antigens, such as complement components C_4 and C_2, plus certain inflammatory proteins, such as tumor necrosis factor (TNF), which are encoded in adjacent areas.

ADHESION MOLECULES

In spite of the known MHC complex consisting of binding of a TCR to the processed antigen, which in turn is bound to the class II molecule of APCs, this is not enough for T-cell activation. One must have additional stimuli that are provided by a series of adhesion molecules on the two cell surfaces.

These molecules are composed of a diverse set of cell-surface glycoproteins and play a pivotal role in mediating cell-to-cell adhesion. Adhesion molecules are divided into four major groups, (a) integrins, (b) selectins, (c) immunoglobulin superfamily, and (d) caherins.

a. Integrins are heterodimers: These are divided into α and β subunits. Depending on the substructure of the β unit, there are five families, but for convenience β_1 and β_2 integrins are involved in leucocyte–endothelial interactions. β_1 integrins, also known as very late activation proteins, are so named because they appear on lymphocytes several days after antigenic stimulation and are composed of a common β chain (CD29) paired with a different α chain. They mediate lymphocyte and monocyte binding to the endothelium receptors called vascular adhesion molecule. β_2 integrins also have a common β chain (CD18), which pairs with different α chains (CD11 a, b, c) to form a number of separate molecules. These two sets of integrins mediate strong binding of leucocytes to the endothelial cell while β_3–β_5 are concerned with binding to extracellular matrix proteins such as fibronectin and vitronectin.
b. Selectins: These molecules are composed of three glycoproteins and are designated by three separate prefixes: E (endothelial), P (platelet), and L (leucocyte). The letters denote the cells on which they were first observed. These groups of selectins bind avidly to carbohydrate molecules on leucocytes and endothelial cells.
c. Immunoglobulin superfamily: The molecules in this family are so called because they contain a common immunoglobulin-like structure. They strengthen the interaction between the T cells and APCs. They include some of the most powerful molecules in the immune system, such as the CD4, CD8, CD2, lymphocyte function antigen (LFA-3 or CD58), and the intercellular adhesion molecules such as ICAM-1 through 3.
d. Cadherins: These molecules are calcium-dependent adhesion molecules and are mainly important in establishing molecular connections between epithelial cells. Their particular importance is during embryonic development.

CYTOKINES

This group of soluble molecules plays an extremely important role in clinical immunology. They are secreted by macrophages and may act as stimulatory or inhibitory signals between cells. Cytokines that initiate chemotaxis of leucocytes are called *chemokines*.

Among the group of cytokines, there are a few of particular interest because of their stimulatory activity. Interleukins 1 (IL-1) and 2 (IL-2) are of particular importance secondary to their role in amplifying the immune response. IL-1 acts on a wide range of cells including T