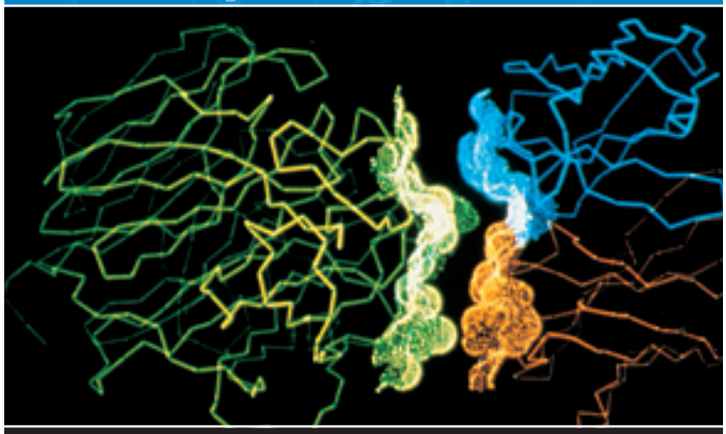
chapter 3

Antigens



Complementarity of Interacting Surfaces of Antibody (left) and Antigen (right)

Substances that can be recognized by the immunoglobulin receptor of B cells, or by the T-cell receptor when complexed with MHC, are called **antigens**. The molecular properties of antigens and the way in which these properties ultimately contribute to immune activation are central to our understanding of the immune system. This chapter describes some of the molecular features of antigens recognized by B or T cells. The chapter also explores the contribution made to immunogenicity by the biological system of the host; ultimately the biological system determines whether a molecule that combines with a B or T cell's antigen-binding receptor can then induce an immune response. Fundamental differences in the way B and T lymphocytes recognize antigen determine which molecular features of an antigen are recognized by each branch of the immune system. These differences are also examined in this chapter.

Immunogenicity Versus Antigenicity

Immunogenicity and antigenicity are related but distinct immunologic properties that sometimes are confused. **Immunogenicity** is the ability to induce a humoral and/or cell-mediated immune response:

B cells + antigen → effector B cells + memory B cells
↓
(plasma cells)

T cells + antigen → effector T cells + memory T cells
↓
(e.g., CTLs, T_Hs)

Although a substance that induces a specific immune response is usually called an antigen, it is more appropriately called an **immunogen.**

Antigenicity is the ability to combine specifically with the final products of the above responses (i.e., antibodies and/or cell-surface receptors). Although all molecules that have the property of immunogenicity also have the property of antigenicity, the reverse is not true. Some small molecules, called *haptens,* are antigenic but incapable, by themselves, of inducing a specific immune response. In other words, they lack immunogenicity.

Factors That Influence Immunogenicity

To protect against infectious disease, the immune system must be able to recognize bacteria, bacterial products, fungi, parasites, and viruses as immunogens. In fact, the immune system actually recognizes particular macromolecules of an infectious agent, generally either proteins or polysaccharides. Proteins are the most potent immunogens, with polysaccharides ranking second. In contrast, lipids and nucleic acids of an infectious agent generally do not serve as immunogens unless they are complexed with proteins or polysaccharides. Immunologists tend to use proteins or polysaccharides as immunogens in most experimental studies of humoral immunity (Table 3-1). For cell-mediated immunity, only proteins and some lipids and glycolipids serve as immunogens. These molecules are not recognized directly. Proteins must first be processed into small peptides and then presented together with MHC molecules on the membrane of a cell before they can be recognized as immunogens. Recent work shows that those lipids and glycolipids that can elicit cell-mediated immunity must also be combined with MHC-like membrane molecules called CD1 (see Chapter 8).

TABLE 3-1 Molecular weight of some common experimental antigens used in immunology

Antigen	Approximate molecular mass (Da)
Bovine gamma globulin (BGG)	150,000
Bovine serum albumin (BSA)	69,000
Flagellin (monomer)	40,000
Hen egg-white lysozyme (HEL)	15,000
Keyhole limpet hemocyanin (KLH)	>2,000,000
Ovalbumin (OVA)	44,000
Sperm whale myoglobin (SWM)	17,000
Tetanus toxoid (TT)	150,000

Immunogenicity is not an intrinsic property of an antigen but rather depends on a number of properties of the particular biological system that the antigen encounters. The next two sections describe the properties that most immunogens share and the contribution that the biological system makes to the expression of immunogenicity.

The Nature of the Immunogen Contributes to Immunogenicity

Immunogenicity is determined, in part, by four properties of the immunogen: its foreignness, molecular size, chemical composition and complexity, and ability to be processed and presented with an MHC molecule on the surface of an antigen-presenting cell or altered self-cell.

FOREIGNNESS

In order to elicit an immune response, a molecule must be recognized as nonself by the biological system. The capacity to recognize nonself is accompanied by tolerance of self, a specific unresponsiveness to self antigens. Much of the ability to tolerate self antigens arises during lymphocyte development, during which immature lymphocytes are exposed to self-components. Antigens that have not been exposed to immature lymphocytes during this critical period may be later recognized as nonself, or foreign, by the immune system. When an antigen is introduced into an organism, the degree of its immunogenicity depends on the degree of its foreignness. Generally, the greater the phylogenetic distance between two species, the greater the structural (and therefore the antigenic) disparity between them.

For example, the common experimental antigen bovine serum albumin (BSA) is not immunogenic when injected into a cow but is strongly immunogenic when injected into a rabbit. Moreover, BSA would be expected to exhibit greater immunogenicity in a chicken than in a goat, which is more closely related to bovines. There are some exceptions to this rule. Some macromolecules (e.g., collagen and cytochrome *c*) have been highly conserved throughout evolution and therefore display very little immunogenicity across diverse species lines. Conversely, some self-components (e.g., corneal tissue and sperm) are effectively sequestered from the immune system, so that if these tissues are injected even into the animal from which they originated, they will function as immunogens.

MOLECULAR SIZE

There is a correlation between the size of a macromolecule and its immunogenicity. The most active immunogens tend to have a molecular mass of 100,000 daltons (Da). Generally, substances with a molecular mass less than 5000–10,000 Da are poor immunogens, although a few substances with a molecular mass less than 1000 Da have proven to be immunogenic.

CHEMICAL COMPOSITION AND HETEROGENEITY

Size and foreignness are not, by themselves, sufficient to make a molecule immunogenic; other properties are needed as well. For example, synthetic homopolymers (polymers composed of a single amino acid or sugar) tend to lack immunogenicity regardless of their size. Studies have shown that copolymers composed of different amino acids or sugars are usually more immunogenic than homopolymers of their constituents. These studies show that chemical complexity contributes to immunogenicity. In this regard it is notable that all four levels of protein organization—primary, secondary, tertiary, and quaternary—contribute to the structural complexity of a protein and hence affect its immunogenicity (Figure 3-1).

LIPIDS AS ANTIGENS

Appropriately presented lipoidal antigens can induce B- and T-cell responses. For the stimulation of B-cell responses, lipids are used as haptens and attached to suitable carrier molecules such as the proteins keyhole limpet hemocyanin (KLH) or bovine serum albumin (BSA). By immunizing with these lipid-protein conjugates it is possible to obtain antibodies that are highly specific for the target lipids. Using this approach, antibodies have been raised against a wide variety of lipid molecules including steroids, complex fatty-acid derivatives, and fat-soluble vitamins such as vitamin E. Such antibodies are of considerable practical importance since many clinical assays for the presence and amounts of medically important lipids are antibody-based. For example, a determination of the levels of a complex group of lipids known as leukotrienes can be useful in evaluating asthma patients. Prednisone, an immunosuppressive steroid, is often given as part of the effort to prevent the rejection of a trans-

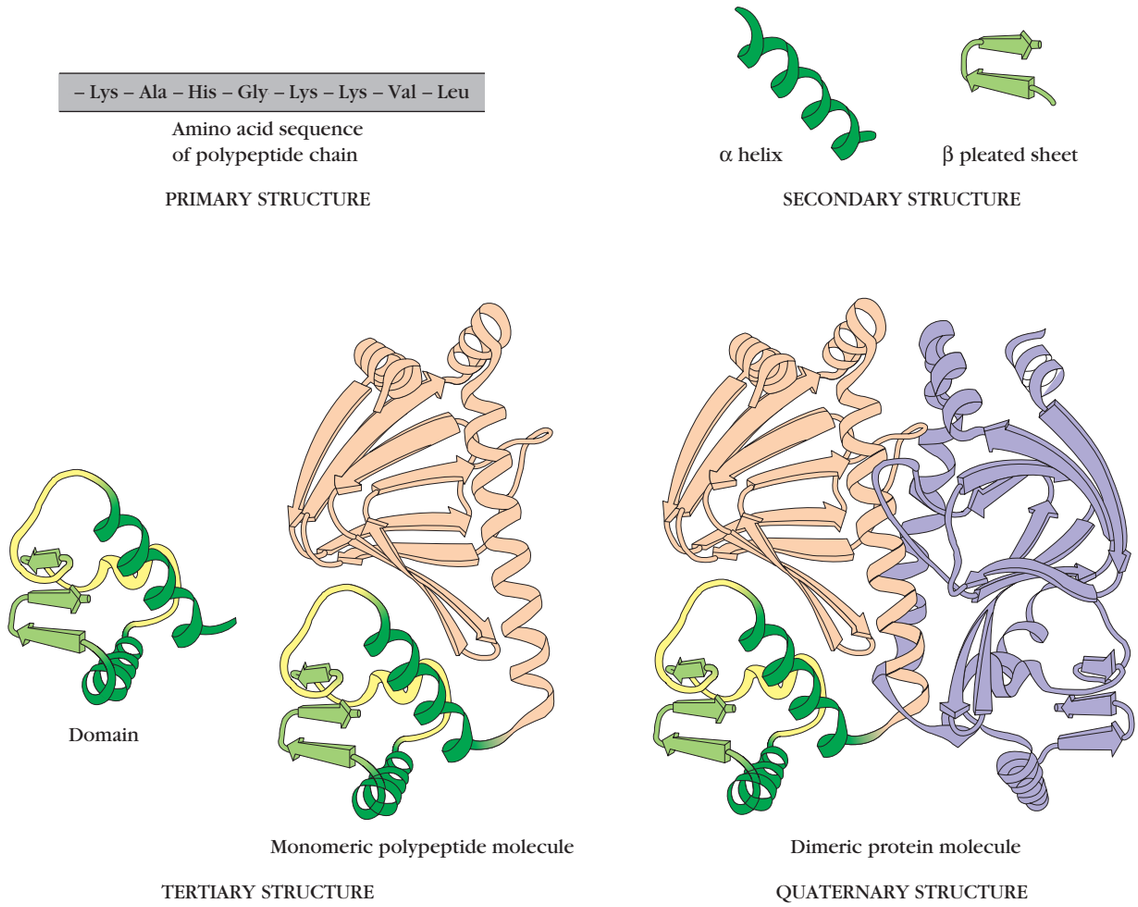
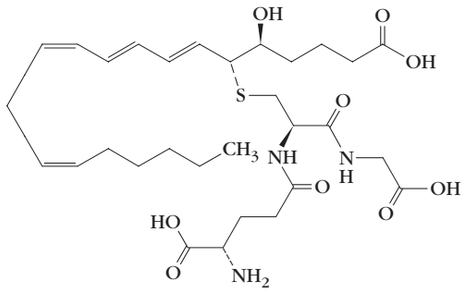
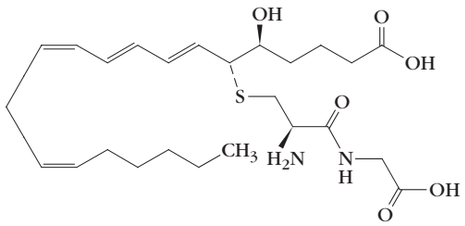
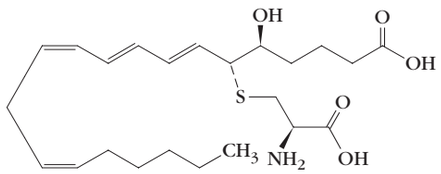
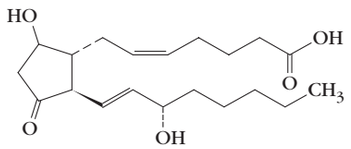


FIGURE 3-1 The four levels of protein organizational structure. The linear arrangement of amino acids constitutes the primary structure. Folding of parts of a polypeptide chain into regular structures (e.g., α helices and β pleated sheets) generates the secondary structure. Tertiary structure refers to the folding of regions between secondary features to give the overall shape of the molecule or parts of it (domains) with specific functional properties. Quaternary structure results from the association of two or more polypeptide chains into a single polymeric protein molecule.

planted organ. The achievement and maintenance of adequate blood levels of this and other immunosuppressive drugs is important to a successful outcome of transplantation, and antibody-based immunoassays are routinely used to make these evaluations. The extraordinary sensitivity and specificity of assays based on the use of anti-lipid antibodies is illustrated by Table 3-2, which shows the specificity of an antibody raised against leukotriene C_4. This antibody allows the detection of as little as 16–32 picograms per ml of leukotriene C_4. Because it has little or no reactivity with similar compounds, such as leukotriene D_4 or leukotriene E_4, it can be used to assay leukotriene C_4 in samples that contain this compound and a variety of other structurally related lipids.

T cells recognize peptides derived from protein antigens when they are presented as peptide-MHC complexes. However, some lipids can also be recognized by T cells. Lipoidal compounds such as glycolipids and some phospholipids can be recognized by T-cell receptors when presented as complexes with molecules that are very much like MHC molecules. These lipid-presenting molecules are members of the CD1 family (see Chapter 8) and are close structural relatives of class I MHC molecules. The lipid molecules recognized by the CD1–T-cell receptor system all appear to share the common feature of a hydrophobic portion and a hydrophilic head group. The hydrophobic portion is a long-chain fatty acid or alcohol and the hydrophilic head group is composed of highly polar groups that often contain carbohydrates. Recognition of lipids is a part of the immune response to some pathogens, and T cells that recognize lipids arising from *Mycobacterium tuberculosis* and *Mycobacterium leprae,* which respectively cause tuberculosis and leprosy, have been isolated from humans infected by these mycobacteria. More about the presentation of lipoidal antigens can be found in Chapter 8.

TABLE 3-2 Specificity of an antibody against a complex lipid

Lipid	Structure	Antibody reactivity* (on scale of 1 to 100)
Leukotriene C_4		100.0
Leukotriene D_4		5.0
Leukotriene E_4		0.5
Prostaglandin D_2		0.001

*The reactivity of the antibody with the immunizing antigen leukotriene C_4 is assigned a value of 100 in arbitrary units.

SUSCEPTIBILITY TO ANTIGEN PROCESSING AND PRESENTATION

The development of both humoral and cell-mediated immune responses requires interaction of T cells with antigen that has been processed and presented together with MHC molecules. Large, insoluble macromolecules generally are more immunogenic than small, soluble ones because the larger molecules are more readily phagocytosed and processed. Macromolecules that cannot be degraded and presented with MHC molecules are poor immunogens. This can be illustrated with polymers of D-amino acids, which are stereoisomers of the naturally occurring L-amino acids. Because the degradative enzymes within antigen-presenting cells can degrade only proteins containing L-amino acids, polymers of D-amino acids cannot be processed and thus are poor immunogens.

The Biological System Contributes to Immunogenicity

Even if a macromolecule has the properties that contribute to immunogenicity, its ability to induce an immune response will depend on certain properties of the biological system that the antigen encounters. These properties include the genotype of the recipient, the dose and route of antigen administration, and the administration of substances, called adjuvants, that increase immune responses.

GENOTYPE OF THE RECIPIENT ANIMAL

The genetic constitution (**genotype**) of an immunized animal influences the type of immune response the animal manifests, as well as the degree of the response. For example, Hugh McDevitt showed that two different inbred strains of

mice responded very differently to a synthetic polypeptide immunogen. After exposure to the immunogen, one strain produced high levels of serum antibody, whereas the other strain produced low levels. When the two strains were crossed, the F_1 generation showed an intermediate response to the immunogen. By backcross analysis, the gene controlling immune responsiveness was mapped to a subregion of the major histocompatibility complex (MHC). Numerous experiments with simple defined immunogens have demonstrated genetic control of immune responsiveness, largely confined to genes within the MHC. These data indicate that MHC gene products, which function to present processed antigen to T cells, play a central role in determining the degree to which an animal responds to an immunogen.

The response of an animal to an antigen is also influenced by the genes that encode B-cell and T-cell receptors and by genes that encode various proteins involved in immune regulatory mechanisms. Genetic variability in all of these genes affects the immunogenicity of a given macromolecule in different animals. These genetic contributions to immunogenicity will be described more fully in later chapters.

IMMUNOGEN DOSAGE AND ROUTE OF ADMINISTRATION

Each experimental immunogen exhibits a particular dose-response curve, which is determined by measuring the immune response to different doses and different administration routes. An antibody response is measured by determining the level of antibody present in the serum of immunized animals. Evaluating T-cell responses is less simple but may be determined by evaluating the increase in the number of T cells bearing TCRs that recognize the immunogen. Some combination of optimal dosage and route of administration will induce a peak immune response in a given animal.

An insufficient dose will not stimulate an immune response either because it fails to activate enough lymphocytes or because, in some cases, certain ranges of low doses can induce a state of immunologic unresponsiveness, or tolerance. The phenomenon of tolerance is discussed in chapters 10 and 21. Conversely, an excessively high dose can also induce tolerance. The immune response of mice to the purified pneumococcal capsular polysaccharide illustrates the importance of dose. A 0.5 mg dose of antigen fails to induce an immune response in mice, whereas a thousand-fold lower dose of the same antigen (5×10^{-4} mg) induces a humoral antibody response. A single dose of most experimental immunogens will not induce a strong response; rather, repeated administration over a period of weeks is usually required. Such repeated administrations, or **boosters,** increase the clonal proliferation of antigen-specific T cells or B cells and thus increase the lymphocyte populations specific for the immunogen.

Experimental immunogens are generally administered parenterally (*para,* around; *enteric,* gut)—that is, by routes other than the digestive tract. The following administration routes are common:

- Intravenous (iv): into a vein
- Intradermal (id): into the skin
- Subcutaneous (sc): beneath the skin
- Intramuscular (im): into a muscle
- Intraperitoneal (ip): into the peritoneal cavity

The administration route strongly influences which immune organs and cell populations will be involved in the response. Antigen administered intravenously is carried first to the spleen, whereas antigen administered subcutaneously moves first to local lymph nodes. Differences in the lymphoid cells that populate these organs may be reflected in the subsequent immune response.

ADJUVANTS

Adjuvants (from Latin *adjuvare,* to help) are substances that, when mixed with an antigen and injected with it, enhance the immunogenicity of that antigen. Adjuvants are often used to boost the immune response when an antigen has low immunogenicity or when only small amounts of an antigen are available. For example, the antibody response of mice to immunization with BSA can be increased fivefold or more if the BSA is administered with an adjuvant. Precisely how adjuvants augment the immune response is not entirely known, but they appear to exert one or more of the following effects (Table 3-3):

- Antigen persistence is prolonged.
- Co-stimulatory signals are enhanced.
- Local inflammation is increased.
- The nonspecific proliferation of lymphocytes is stimulated.

Aluminum potassium sulfate (alum) prolongs the persistence of antigen. When an antigen is mixed with alum, the salt precipitates the antigen. Injection of this alum precipitate results in a slower release of antigen from the injection site, so that the effective time of exposure to the antigen increases from a few days without adjuvant to several weeks with the adjuvant. The alum precipitate also increases the size of the antigen, thus increasing the likelihood of phagocytosis.

Water-in-oil adjuvants also prolong the persistence of antigen. A preparation known as **Freund's incomplete adjuvant** contains antigen in aqueous solution, mineral oil, and an emulsifying agent such as mannide monooleate, which disperses the oil into small droplets surrounding the antigen; the antigen is then released very slowly from the site of injection. This preparation is based on **Freund's complete adjuvant,** the first deliberately formulated highly effective adjuvant, developed by Jules Freund many years ago and containing heat-killed *Mycobacteria* as an additional ingredient. Muramyl dipeptide, a component of the mycobacterial cell wall, activates macrophages, making

TABLE 3-3 Postulated mode of action of some commonly used adjuvants

	POSTULATED MODE OF ACTION			
Adjuvant	**Prolongs antigen persistence**	**Enhances co-stimulatory signal**	**Induces granuloma formation**	**Stimulates lymphocytes nonspecifically**
Freund's incomplete adjuvant	+	+	+	−
Freund's complete adjuvant	+	++	++	−
Aluminum potassium sulfate (alum)	+	?	+	−
Mycobacterium tuberculosis	−	?	+	−
Bordetella pertussis	−	?	−	+
Bacterial lipopolysaccharide (LPS)	−	+	−	+
Synthetic polynucleotides (poly IC/poly AU)	−	?	−	+

Freund's complete adjuvant far more potent than the incomplete form. Activated macrophages are more phagocytic than unactivated macrophages and express higher levels of class II MHC molecules and the membrane molecules of the B7 family. The increased expression of class II MHC increases the ability of the antigen-presenting cell to present antigen to T_H cells. B7 molecules on the antigen-presenting cell bind to CD28, a cell-surface protein on T_H cells, triggering co-stimulation, an enhancement of the T-cell immune response. Thus, antigen presentation and the requisite co-stimulatory signal usually are increased in the presence of adjuvant.

Alum and Freund's adjuvants also stimulate a local, chronic inflammatory response that attracts both phagocytes and lymphocytes. This infiltration of cells at the site of the adjuvant injection often results in formation of a dense, macrophage-rich mass of cells called a **granuloma.** Because the macrophages in a granuloma are activated, this mechanism also enhances the activation of T_H cells.

Other adjuvants (e.g., synthetic polyribonucleotides and bacterial lipopolysaccharides) stimulate the nonspecific proliferation of lymphocytes and thus increase the likelihood of antigen-induced clonal selection of lymphocytes.

Epitopes

As mentioned in Chapter 1, immune cells do not interact with, or recognize, an entire immunogen molecule; instead, lymphocytes recognize discrete sites on the macromolecule called **epitopes,** or **antigenic determinants.** Epitopes are the immunologically active regions of an immunogen that bind to antigen-specific membrane receptors on lymphocytes or to secreted antibodies. Studies with small antigens have revealed that B and T cells recognize different epitopes on the same antigenic molecule. For example, when mice were immunized with glucagon, a small human hormone of 29 amino acids, antibody was elicited to epitopes in the amino-terminal portion, whereas the T cells responded only to epitopes in the carboxyl-terminal portion.

Lymphocytes may interact with a complex antigen on several levels of antigen structure. An epitope on a protein antigen may involve elements of the primary, secondary, tertiary, and even quaternary structure of the protein (see Figure 3-1). In polysaccharides, branched chains are commonly present, and multiple branches may contribute to the conformation of epitopes.

The recognition of antigens by T cells and B cells is fundamentally different (Table 3-4). B cells recognize soluble antigen when it binds to their membrane-bound antibody. Because B cells bind antigen that is free in solution, the epitopes they recognize tend to be highly accessible sites on the exposed surface of the immunogen. As noted previously, most T cells recognize only peptides combined with MHC molecules on the surface of antigen-presenting cells and altered self-cells; T-cell epitopes, as a rule, cannot be considered apart from their associated MHC molecules.

Properties of B-Cell Epitopes Are Determined by the Nature of the Antigen-Binding Site

Several generalizations have emerged from studies in which the molecular features of the epitope recognized by B cells have been established.

The ability to function as a B-cell epitope is determined by the nature of the antigen-binding site on the antibody molecules displayed by B cells. Antibody binds to an epitope by weak noncovalent interactions, which operate only over short distances. For a strong bond, the antibody's binding site and the epitope must have complementary shapes that place the interacting groups near each other. This requirement poses some restriction on the properties of the epitope. The size of the epitope recognized by a B cell can be no larger than the size of the antibody's binding site. For any given antigen-antibody reaction, the shape of the epitope that can be recognized by the antibody is determined by the shape assumed by

TABLE 3-4 Comparison of antigen recognition by T cells and B cells

Characteristic	B cells	T cells
Interaction with antigen	Involves binary complex of membrane Ig and Ag	Involves ternary complex of T-cell receptor, Ag, and MHC molecule
Binding of soluble antigen	Yes	No
Involvement of MHC molecules	None required	Required to display processed antigen
Chemical nature of antigens	Protein, polysaccharide, lipid	Mostly proteins, but some lipids and glycolipids presented on MHC-like molecules
Epitope properties	Accessible, hydrophilic, mobile peptides containing sequential or nonsequential amino acids	Internal linear peptides produced by processing of antigen and bound to MHC molecules

the sequences of amino acids in the binding site and the chemical environment that they produce.

Smaller ligands such as carbohydrates, small oligonucleotides, peptides, and haptens often bind within a deep pocket of an antibody. For example, angiotensin II, a small octapeptide hormone, binds within a deep and narrow groove (725 $Å^2$) of a monoclonal antibody specific for the hormone (Figure 3-2). Within this groove, the bound peptide hormone folds into a compact structure with two turns, which brings its amino (N-terminal) and carboxyl (C-terminal) termini close together. All eight amino acid residues of the octapeptide are in van der Waals contact with 14 residues of the antibody's groove.

A quite different picture of epitope structure emerges from x-ray crystallographic analyses of monoclonal antibodies bound to globular protein antigens such as hen egg-white lysozyme (HEL) and neuraminidase (an envelope glycoprotein of influenza virus). These antibodies make contact with the antigen across a large flat face (Figure 3-3). The interacting face between antibody and epitope is a flat or undulating surface in which protrusions on the epitope or antibody are matched by corresponding depressions on the antibody or epitope. These studies have revealed that 15–22 amino acids on the surface of the antigen make contact with a similar number of residues in the antibody's binding site; the surface area of this large complementary interface is between 650 $Å^2$ and 900 $Å^2$. For these globular protein antigens, then, the shape of the epitope is entirely determined by the tertiary conformation of the native protein.

Thus, globular protein antigens and small peptide antigens interact with antibody in different ways (Figure 3-4). Typically, larger areas of protein antigens are engaged by the antibody binding site. In contrast, a small peptide such as angiotensin II can fold into a compact structure that occupies less space and fits into a pocket or cleft of the binding site. This pattern is not unique to small peptides; it extends to the binding of low-molecular-weight antigens of various chemical types. However, these differences between the binding of small and large antigenic determinants do not reflect fundamental differences in the regions of the antibody molecule that make up the binding site. Despite differences in the binding patterns of small haptens and large antigens, Chapter 4 will show that all antibody binding sites are assembled from the same regions of the antibody molecule—namely, parts of the variable regions of its polypeptide chains.

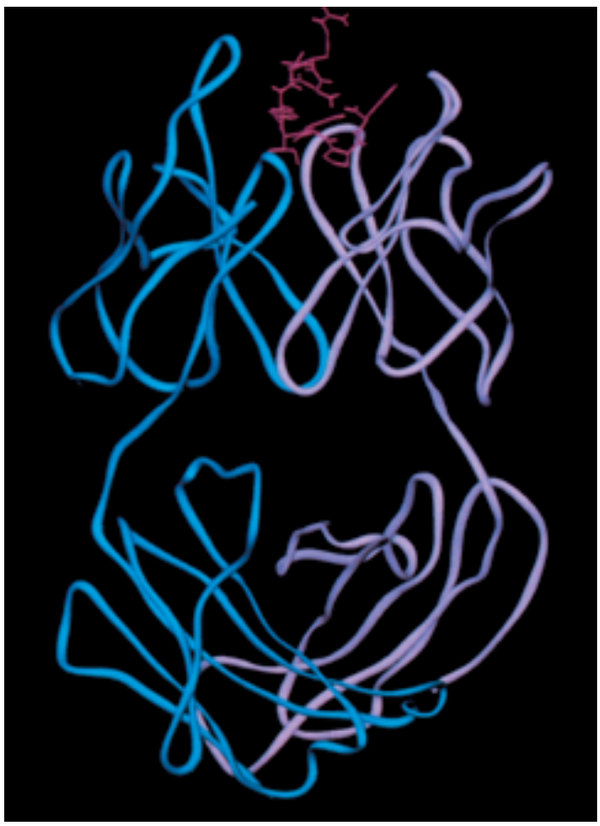


FIGURE 3-2 Three-dimensional structure of an octapeptide hormone (angiotensin II) complexed with a monoclonal antibody Fab fragment, the antigen-binding unit of the antibody molecule. The angiotensin II peptide is shown in red, the heavy chain in blue, and the light chain in purple. *[From K. C. Garcia et al., 1992,* Science ***257:****502.]*

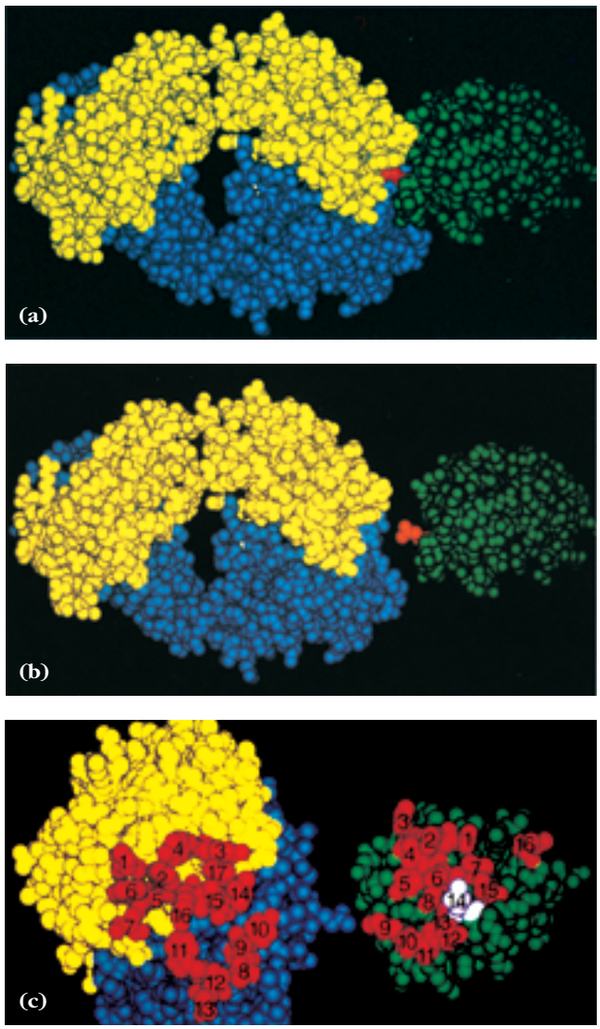


FIGURE 3-3 (a) Model of interaction between hen egg-white lysozyme (HEL) and Fab fragment of anti-HEL antibody based on x-ray diffraction analysis. HEL is shown in green, the Fab heavy chain in blue, and the Fab light chain in yellow. A glutamine residue of lysozyme (red) fits into a pocket in the Fab fragment. (b) Representation of HEL and the Fab fragment when pulled apart showing complementary surface features. (c) View of the interacting surfaces of the Fab fragment and HEL obtained by rotating each of the molecules. The contacting residues are numbered and shown in red with the protruding glutamine (#14) in HEL now shown in white. *[From A. G. Amit et al., 1986,* Science ***233:*** *747.]*

The B-cell epitopes on native proteins generally are composed of hydrophilic amino acids on the protein surface that are topographically accessible to membrane-bound or free antibody. A B-cell epitope must be accessible in order to be able to bind to an antibody; in general, protruding regions on the surface of the protein are the most likely to be recognized as epitopes, and these regions are usually composed of predominantly hydrophilic amino acids. Amino acid sequences that are hidden within the interior of a protein often consist of predominantly hydrophobic amino acids, and cannot function as B-cell epitopes unless the protein is first denatured. In the crystallized antigen-antibody complexes analyzed to date, the interface between antibody and antigen shows numerous complementary protrusions and depressions (Figure 3-5). Between 15 and 22 amino acids on the antigen contact the antibody by 75–120 hydrogen bonds as well as by ionic and hydrophobic interactions.

B-cell epitopes can contain sequential or nonsequential amino acids. Epitopes may be composed of sequential contiguous residues along the polypeptide chain or nonsequential residues from segments of the chain brought together by the folded conformation of an antigen. Most antibodies elicited by globular proteins bind to the protein only when it is in its native conformation. Because denaturation of such antigens usually changes the structure of their epitopes, antibodies to the native protein do not bind to the denatured protein.

Five distinct **sequential epitopes,** each containing six to eight contiguous amino acids, have been found in sperm whale myoglobin. Each of these epitopes is on the surface of the molecule at bends between the α-helical regions (Figure 3-6a). Sperm whale myoglobin also contains several **nonsequential epitopes,** or **conformational determinants.** The residues that constitute these epitopes are far apart in the primary amino acid sequence but close together in the tertiary structure of the molecule. Such epitopes depend on the

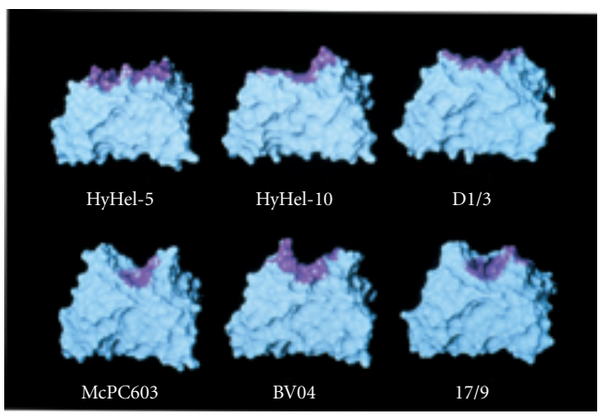


FIGURE 3-4 Models of the variable domains of six Fab fragments with their antigen-binding regions shown in purple. The top three antibodies are specific for lysozyme, a large globular protein. The lower three antibodies are specific for smaller molecules or very small segments of macromolecules: McPC603 for phosphocholine; BV04 for a small segment of a single-stranded DNA molecule; and 17/9 for a peptide from hemagglutinin, an envelope protein of influenza virus. In general, the binding sites for small molecules are deep pockets, whereas binding sites for large proteins are flatter, more undulating surfaces. *[From I. A. Wilson and R. L. Stanfield, 1993,* Curr. Opin. Struc. Biol. ***3:*** *113.]*

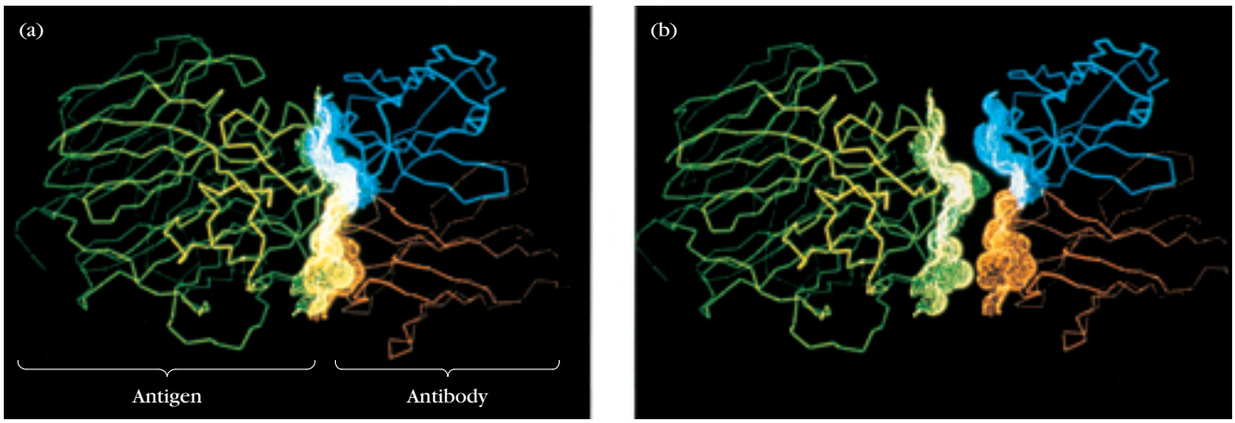


FIGURE 3-5 Computer simulation of an interaction between antibody and influenza virus antigen, a globular protein. (a) The antigen (yellow) is shown interacting with the antibody molecule; the variable region of the heavy chain is red, and the variable region of the light chain is blue. (b) The complementarity of the two molecules is revealed by separating the antigen from the antibody by 8 Å. *[Based on x-ray crystallography data collected by P. M. Colman and W. R. Tulip. From G. J. V. H. Nossal, 1993,* Sci. Am. ***269(3):****22.]*

VISUALIZING CONCEPTS

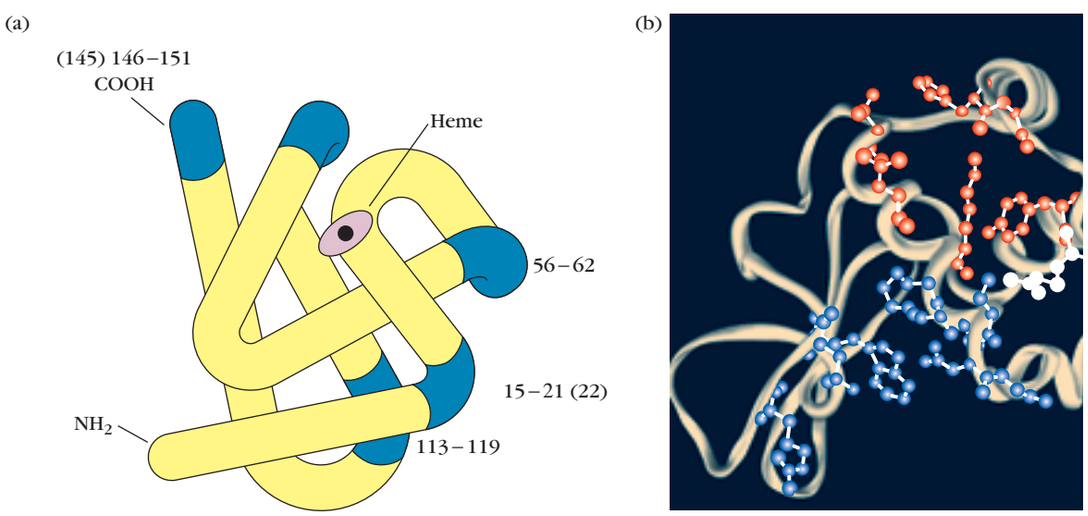


FIGURE 3-6 Protein antigens usually contain both sequential and nonsequential B-cell epitopes. (a) Diagram of sperm whale myoglobin showing locations of five sequential B-cell epitopes (blue). (b) Ribbon diagram of hen egg-white lysozyme showing residues that compose one nonsequential (conformational) epitope. Residues that contact antibody light chains, heavy chains, or both are shown in red, blue, and white, respectively. These residues are widely spaced in the amino acid sequence but are brought into proximity by folding of the protein. *[Part (a) adapted from M. Z. Atassi and A. L. Kazim. 1978,* Adv. Exp. Med. Biol. ***98:****9; part (b) from W. G. Laver et al., 1990,* Cell ***61:****554.]*

native protein conformation for their topographical structure. One well-characterized nonsequential epitope in hen egg-white lysozyme (HEL) is shown in Figure 3-6b. Although the amino acid residues that compose this epitope of HEL are far apart in the primary amino acid sequence, they are brought together by the tertiary folding of the protein.

Sequential and nonsequential epitopes generally behave differently when a protein is denatured, fragmented, or reduced. For example, appropriate fragmentation of sperm whale myoglobin can yield five fragments, each retaining one sequential epitope, as demonstrated by the observation that antibody can bind to each fragment. On the other hand, fragmentation of a protein or reduction of its disulfide bonds often destroys nonsequential epitopes. For example, HEL has four intrachain disulfide bonds, which determine the final protein conformation (Figure 3-7a). Many antibodies to HEL recognize several epitopes, and each of eight different epitopes have been recognized by a distinct antibody. Most of these epitopes are conformational determinants dependent on the overall structure of the protein. If the intrachain disulfide bonds of HEL are reduced with mercaptoethanol, the nonsequential epitopes are lost; for this reason, antibody to native HEL does not bind to reduced HEL.

The inhibition experiment shown in Figure 3-7 nicely demonstrates this point. An antibody to a conformational determinant, in this example a peptide loop present in native HEL, was able to bind the epitope only if the disulfide bond that maintains the structure of the loop was intact. Information about the structural requirements of the antibody combining site was obtained by examining the ability of structural relatives of the natural antigen to bind to that antibody. If a structural relative has the critical epitopes present in the natural antigen, it will bind to the antibody combining site, thereby blocking its occupation by the natural antigen. In this inhibition assay, the ability of the closed loop to inhibit binding showed that the closed loop was sufficiently

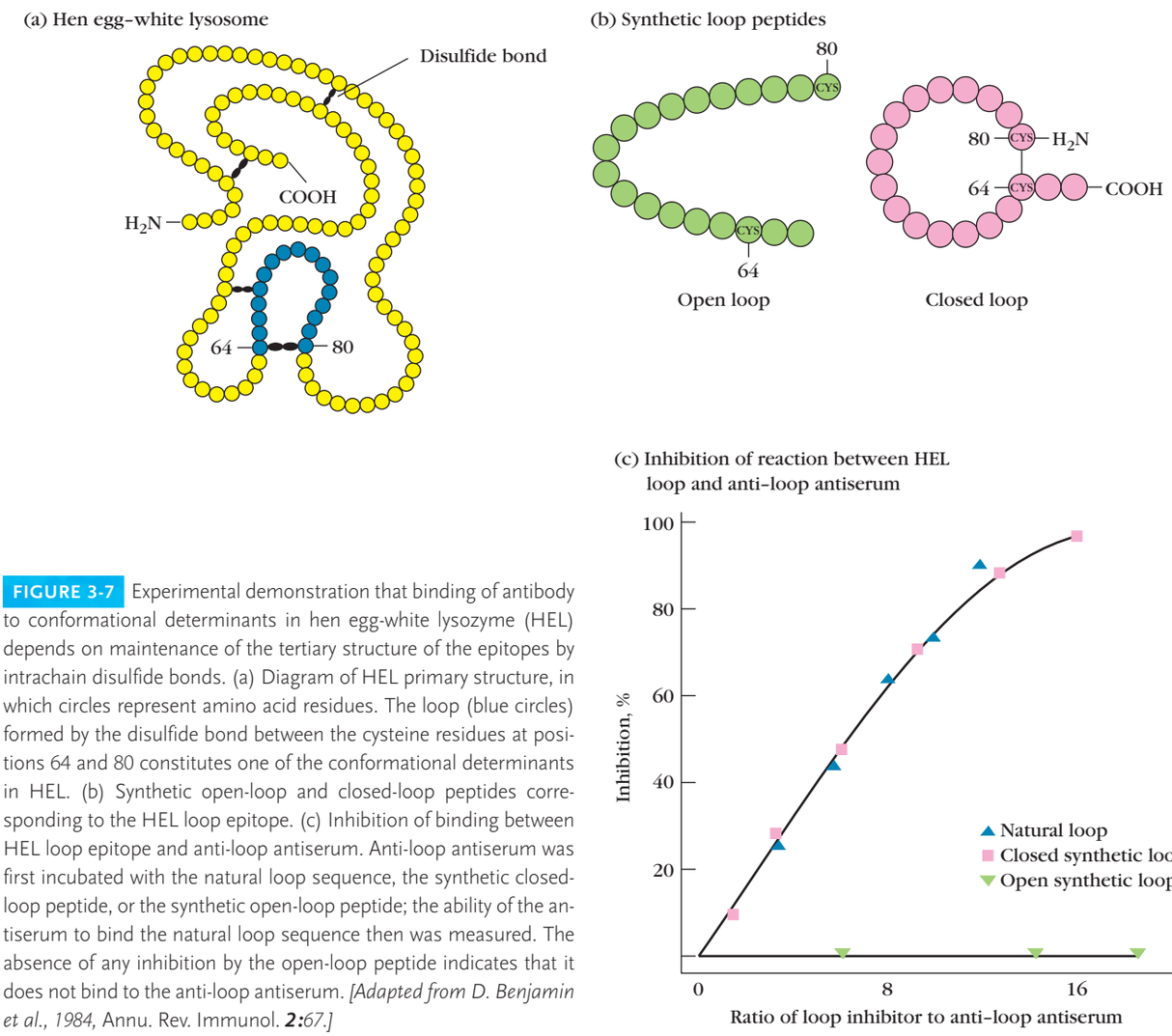


FIGURE 3-7 Experimental demonstration that binding of antibody to conformational determinants in hen egg-white lysozyme (HEL) depends on maintenance of the tertiary structure of the epitopes by intrachain disulfide bonds. (a) Diagram of HEL primary structure, in which circles represent amino acid residues. The loop (blue circles) formed by the disulfide bond between the cysteine residues at positions 64 and 80 constitutes one of the conformational determinants in HEL. (b) Synthetic open-loop and closed-loop peptides corresponding to the HEL loop epitope. (c) Inhibition of binding between HEL loop epitope and anti-loop antiserum. Anti-loop antiserum was first incubated with the natural loop sequence, the synthetic closed-loop peptide, or the synthetic open-loop peptide; the ability of the antiserum to bind the natural loop sequence then was measured. The absence of any inhibition by the open-loop peptide indicates that it does not bind to the anti-loop antiserum. *[Adapted from D. Benjamin et al., 1984,* Annu. Rev. Immunol. ***2:****67.]*

similar to HEL to be recognized by antibody to native HEL. Even though the open loop had the same sequence of amino acids as the closed loop, it lacked the epitopes recognized by the antibody and therefore was unable to block binding of HEL.

B-cell epitopes tend to be located in flexible regions of an immunogen and display site mobility. John A. Tainer and his colleagues analyzed the epitopes on a number of protein antigens (myohemerytherin, insulin, cytochrome *c,* myoglobin, and hemoglobin) by comparing the positions of the known B-cell epitopes with the mobility of the same residues. Their analysis revealed that the major antigenic determinants in these proteins generally were located in the most mobile regions. These investigators proposed that site mobility of epitopes maximizes complementarity with the antibody's binding site, permitting an antibody to bind with an epitope that it might bind ineffectively if it were rigid. However, because of the loss of entropy due to binding to a flexible site, the binding of antibody to a flexible epitope is generally of lower affinity than the binding of antibody to a rigid epitope.

Complex proteins contain multiple overlapping B-cell epitopes, some of which are immunodominant. For many years, it was dogma in immunology that each globular protein had a small number of epitopes, each confined to a highly accessible region and determined by the overall conformation of the protein. However, it has been shown more recently that most of the surface of a globular protein is potentially antigenic. This has been demonstrated by comparing the antigen-binding profiles of different monoclonal antibodies to various globular proteins. For example, when 64 different monoclonal antibodies to BSA were compared for their ability to bind to a panel of 10 different mammalian albumins, 25 different overlapping antigen-binding profiles emerged, suggesting that these 64 different antibodies recognized a minimum of 25 different epitopes on BSA. Similar findings have emerged for other globular proteins, such as myoglobin and HEL.

The surface of a protein, then, presents a large number of potential antigenic sites. The subset of antigenic sites on a given protein that is recognized by the immune system of an animal is much smaller than the potential antigenic repertoire, and it varies from species to species and even among individual members of a given species. Within an animal, certain epitopes of an antigen are recognized as immunogenic, but others are not. Furthermore, some epitopes, called **immunodominant,** induce a more pronounced immune response than other epitopes in a particular animal. It is highly likely that the intrinsic topographical properties of the epitope as well as the animal's regulatory mechanisms influence the immunodominance of epitopes.

Antigen-Derived Peptides Are the Key Elements of T-Cell Epitopes

Studies by P. G. H. Gell and Baruj Benacerraf in 1959 suggested that there was a qualitative difference between the T-cell and the B-cell response to protein antigens. Gell and Benacerraf compared the humoral (B-cell) and cell-mediated (T-cell) responses to a series of native and denatured protein antigens (Table 3-5). They found that when primary immunization was with a native protein, only native protein, not denatured protein, could elicit a secondary antibody (humoral) response. In contrast, both native and denatured protein could elicit a secondary cell-mediated response. The finding that a secondary response mediated by T cells was induced by denatured protein, even when the primary immunization had been with native protein, initially puzzled immunologists. In the 1980s, however, it became clear that T cells do not recognize soluble native antigen but rather recognize antigen that has been processed into **antigenic peptides,** which are presented in combination with MHC molecules. For this reason, destruction of the conformation of a protein by denaturation does not affect its T-cell epitopes.

Because the T-cell receptor does not bind free peptides, experimental systems for studying T-cell epitopes must include antigen-presenting cells or target cells that can display the peptides bound to an MHC molecule.

Antigenic peptides recognized by T cells form trimolecular complexes with a T-cell receptor and an MHC molecule (Figure 3-8). The structures of TCR-peptide-MHC trimolecular complexes have been determined by x-ray crystallography and are described in Chapter 9. These structural studies of class I or class II MHC molecules crystallized with known T-cell antigenic peptides has shown that the peptide binds to a

TABLE 3-5 Antigen recognition by T and B lymphocytes reveals qualitative differences

		SECONDARY IMMUNE RESPONSE	
Primary immunization	**Secondary immunization**	**Antibody production**	**Cell-mediated T_{DTH} response***
Native protein	Native protein	+	+
Native protein	Denatured protein	−	+

*T_{DTH} is a subset of $CD4^+$ T_H cells that mediate a cell-mediated response called delayed-type hypersensitivity (see Chapter 14).

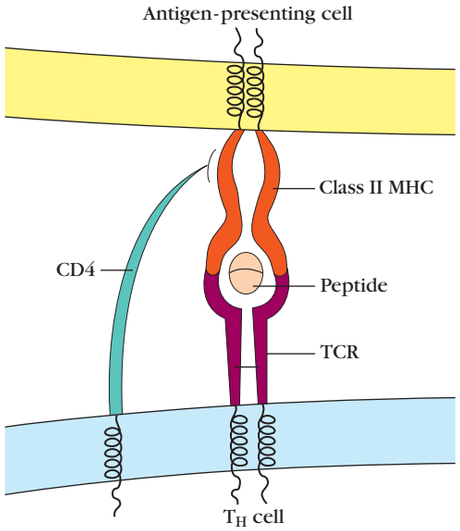


FIGURE 3-8 Schematic diagram of the ternary complex formed between a T-cell receptor (TCR) on a T_H cell, an antigen, and a class II MHC molecule. Antigens that are recognized by T cells yield peptides that interact with MHC molecules to form a peptide-MHC complex that is recognized by the T-cell receptor. As described in later chapters, the coreceptor, CD4, on T_H cells also interacts with MHC molecules. T_C cells form similar ternary complexes with class I MHC molecules on target cells however, these cells bear MHC-interacting CD8 coreceptors.

cleft in the MHC molecule (see Figure 7-8). Unlike B-cell epitopes, which can be viewed strictly in terms of their ability to interact with antibody, T-cell epitopes must be viewed in terms of their ability to interact with both a T-cell receptor and an MHC molecule.

The binding of an MHC molecule to an antigenic peptide does not have the fine specificity of the interaction between an antibody and its epitope. Instead, a given MHC molecule can selectively bind a variety of different peptides. For example, the class II MHC molecule designated IA^d can bind peptides from ovalbumin (residues 323–339), hemagglutinin (residues 130–142), and lambda repressor (residues 12–26). Studies revealing structural features, or motifs, common to different peptides that bind to a single MHC molecule are described in Chapter 7.

Antigen processing is required to generate peptides that interact specifically with MHC molecules. As mentioned in Chapter 1, endogenous and exogenous antigens are usually processed by different intracellular pathways (see Figure 1-9). Endogenous antigens are processed into peptides within the cytoplasm, while exogenous antigens are processed by the endocytic pathway. The details of antigen processing and presentation are described in Chapter 8.

Epitopes recognized by T cells are often internal. T cells tend to recognize internal peptides that are exposed by processing within antigen-presenting cells or altered self-cells. J. Rothbard analyzed the tertiary conformation of hen egg-white lysozyme and sperm whale myoglobin to determine which amino acids protruded from the natural molecule. He then mapped the major T-cell epitopes for both proteins and found that, in each case, the T-cell epitopes tended to be on the "inside" of the protein molecule (Figure 3-9).

Haptens and the Study of Antigenicity

The pioneering work of Karl Landsteiner in the 1920s and 1930s created a simple, chemically defined system for studying the binding of an individual antibody to a unique epitope

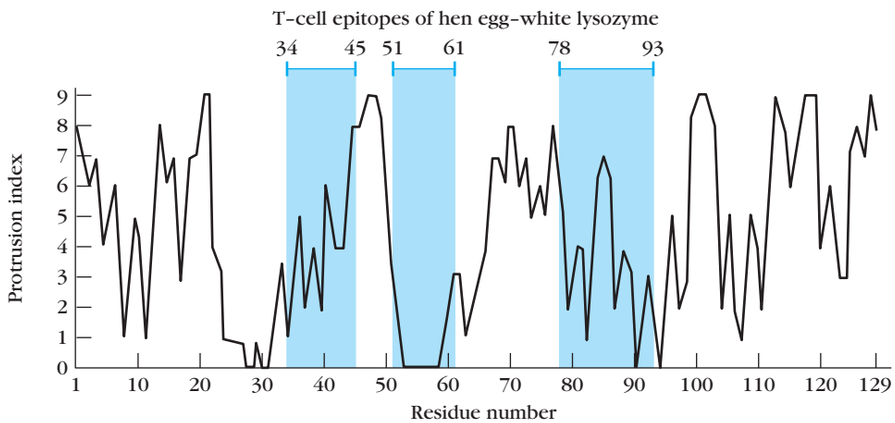


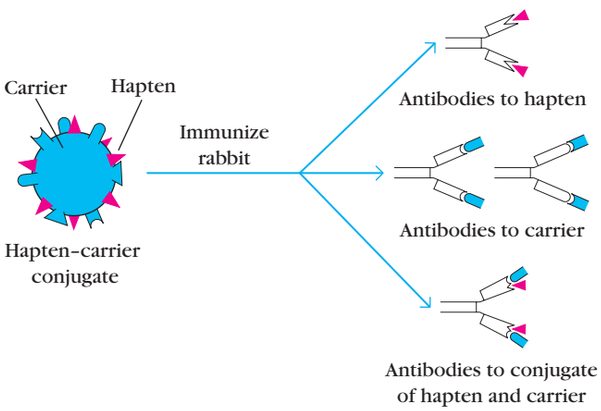
FIGURE 3-9 Experimental evidence that T_H cells tend to recognize internal peptides of antigens. This plot shows the relative protrusion of amino acid residues in the tertiary conformation of hen egg-white lysozyme. The known T-cell epitopes in HEL are indicated by the blue bars at the top. Notice that, in general, the amino acid residues that correspond to the T-cell epitopes exhibit less overall protrusion. In contrast, note that the B-cell epitope consisting of residues 64–80, which form a conformational determinant in native HEL that is recognized by antibody (see Figure 3-7), exhibit greater overall protrusion. *[From J. Rothbard et al., 1987,* Mod. Trends Hum. Leuk., *vol. 7.]*

on a complex protein antigen. Landsteiner employed various **haptens,** small organic molecules that are antigenic but not immunogenic. Chemical coupling of a hapten to a large protein, called a **carrier,** yields an immunogenic **hapten-carrier conjugate.** Animals immunized with such a conjugate produce antibodies specific for (1) the hapten determinant, (2) unaltered epitopes on the carrier protein, and (3) new epitopes formed by combined parts of both the hapten and carrier (Figure 3-10). By itself, a hapten cannot function as an immunogenic epitope. But when multiple molecules of a single hapten are coupled to a carrier protein (or nonimmunogenic homopolymer), the hapten becomes accessible to the immune system and can function as an immunogen.

The beauty of the hapten-carrier system is that it provides immunologists with a chemically defined determinant that can be subtly modified by chemical means to determine the effect of various chemical structures on immune specificity. In his studies, Landsteiner immunized rabbits with a hapten-carrier conjugate and then tested the reactivity of the rabbit's immune sera with that hapten and with closely related haptens coupled to a different carrier protein. He was thus able to measure, specifically, the reaction of the antihapten antibodies in the immune serum and not that of antibodies to the original carrier epitopes. Landsteiner tested whether an antihapten antibody could bind to other haptens having a slightly different chemical structure. If a reaction occurred, it was called a **cross-reaction.** By observing which hapten modifications prevented or permitted cross-reactions, Landsteiner was able to gain insight into the specificity of antigen-antibody interactions.

Using various derivatives of aminobenzene as haptens, Landsteiner found that the overall configuration of a hapten plays a major role in determining whether it can react with a given antibody. For example, antiserum from rabbits immunized with aminobenzene or one of its carboxyl derivatives (*o*-aminobenzoic acid, *m*-aminobenzoic acid, or *p*-aminobenzoic acid) coupled to a carrier protein reacted only with the original immunizing hapten and did not cross-react with any of the other haptens (Table 3-6). In contrast, if the overall configuration of the hapten was kept the same and the hapten was modified in the para position with various nonionic derivatives, then the antisera showed various degrees of cross-reactivity. Landsteiner's work not only demonstrated the specificity of the immune system, but also demonstrated the enormous diversity of epitopes that the immune system is capable of recognizing.

Many biologically important substances, including drugs, peptide hormones, and steroid hormones, can function as haptens. Conjugates of these haptens with large protein carriers can be used to produce hapten-specific antibodies. These antibodies are useful for measuring the presence of various substances in the body. For instance, the original home pregnancy test kit employed antihapten antibodies to determine whether a woman's urine contained human chorionic gonadotropin (HCG), which is a sign of pregnancy. However, as shown in the Clinical Focus, the formation of drug-protein conjugates in the body can produce drug allergies that may be life-threatening.



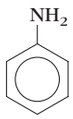
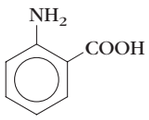
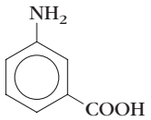
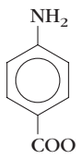
Injection with:	Antibodies formed:
Hapten (DNP)	None
Protein carrier (BSA)	Anti-BSA
Hapten–carrier conjugate (DNP-BSA)	Anti-DNP (major) Anti-BSA (minor) Anti-DNP/BSA (minor)

FIGURE 3-10 A hapten-carrier conjugate contains multiple copies of the hapten—a small nonimmunogenic organic compound such as dinitrophenol (DNP)—chemically linked to a large protein carrier such as bovine serum albumin (BSA). Immunization with DNP alone elicits no anti-DNP antibodies, but immunization with DNP-BSA elicits three types of antibodies. Of these, anti-DNP antibody is predominant, indicating that in this case the hapten is the immunodominant epitope in a hapten-carrier conjugate, as it often is in such conjugates.

Pattern-Recognition Receptors

The receptors of adaptive and innate immunity differ. Antibodies and T-cell receptors, the receptors of adaptive immunity, recognize details of molecular structure and can discriminate with exquisite specificity between antigens featuring only slight structural differences. The receptors of innate immunity recognize broad structural motifs that are highly conserved within microbial species but are generally absent from the host. Because they recognize particular overall molecular patterns, such receptors are called **pattern-recognition receptors (PRRs).** Patterns recognized by this type of receptor include combinations of sugars, certain proteins, particular lipid-bearing molecules, and some nucleic acid motifs. Typically, the ability of pattern-recognition receptors to distinguish between self and nonself is perfect because the molecular pattern targeted by the receptor is produced only by the pathogen and never by the host. This contrasts sharply with the occasional recognition of self

TABLE 3-6 Reactivity of antisera with various haptens

	REACTIVITY WITH			
				
Antiserum against	**Aminobenzene (aniline)**	***o*-Aminobenzoic acid**	***m*-Aminobenzoic acid**	***p*-Aminobenzoic acid**
Aminobenzene	+	0	0	0
o-Aminobenzoic acid	0	+	0	0
m-Aminobenzoic acid	0	0	+	0
p-Aminobenzoic acid	0	0	0	+

KEY: 0 = no reactivity; + = strong reactivity

SOURCE: Based on K. Landsteiner, 1962, *The Specificity of Serologic Reactions,* Dover Press. Modified by J. Klein, 1982, *Immunology: The Science of Self-Nonself Discrimination,* John Wiley.

antigens by receptors of adaptive immunity, which can lead to autoimmune disorders. Like antibodies and T-cell receptors, pattern-recognition receptors are proteins. However, the genes that encode PRRs are present in the germline of the organism. In contrast, the genes that encode the enormous diversity of antibodies and TCRs are not present in the germline. They are generated by an extraordinary process of genetic recombination that is discussed in Chapter 5.

Many different pattern-recognition receptors have been identified and several examples appear in Table 3-7. Some are present in the bloodstream and tissue fluids as soluble circulating proteins and others are on the membrane of cells such as macrophages, neutrophils, and dendritic cells. Mannose-binding lectin (MBL) and C-reactive protein (CRP) are soluble pattern receptors that bind to microbial surfaces and promote their opsonization. Both of these receptors also have the ability to activate the complement system when they are bound to the surface of microbes, thereby making the invader a likely target of complement-mediated lysis. Yet another soluble receptor of the innate immune system, lipopolysaccharide-binding protein, is an important part of the system that recognizes and signals a response to lipopolysaccharide, a component of the outer cell wall of gram-negative bacteria.

Pattern-recognition receptors found on the cell membrane include scavenger receptors and the toll-like receptors. Scavenger receptors (SRs) are present on macrophages and many types of dendritic cells, and are involved in the binding and internalization of gram-positive and gram-negative bacteria, as well as the phagocytosis of apoptotic host cells. The exact roles and mechanisms of action of the many types of scavenger receptors known to date are under active investigation. The toll-like receptors (TLRs) are important in recognizing many microbial patterns. This family of proteins is ancient—toll-like receptors mediate the recognition and generation of defensive responses to pathogens in organisms as widely separated in evolutionary history as humans and flies. Typically, signals transduced through the TLRs cause transcriptional activation and the synthesis and secretion of cytokines, which promote inflammatory responses that bring macrophages and neutrophils to sites of inflammation.

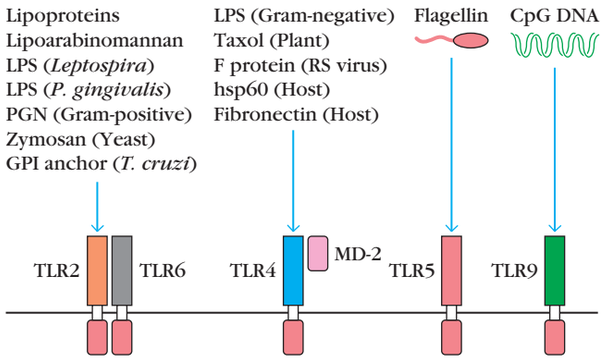


FIGURE 3-11 Location and targets of some pattern-recognition receptors. Many pattern-recognition receptors are extracellular and target microbes or microbial components in the bloodstream and tissue fluids, causing their lysis or marking them for removal by phagocytes. Other pattern-recognition receptors are present on the cell membrane and bind to a broad variety of microbes or microbial products. Engagement of these receptors triggers signaling pathways that promote inflammation or, in the case of the scavenger receptors, phagocytosis or endocytosis. dsRNA = double stranded RNA; LPS = lipopolysaccharide. *[S. Akira et al., 2001,* Nature Immunology ***2:****675.]*

TABLE 3-7 Receptors of innate and adaptive immunity

Characteristic	Innate immunity	Adaptive immunity
Specificity	Specific for conserved molecular patterns or types	Specific for details of antigen structure
Self/nonself discrimination	Perfect: evolutionarily selected to distinguish phylogenetic differences. Never recognizes self.	Excellent: but imperfect. Occasional reaction with self antigens

RECEPTORS OF THE ADAPTIVE IMMUNE SYSTEM

Receptor (location)	Target (source)	Effect of recognition
Antibody (B-cell membrane, blood, tissue fluids)	Specific components of pathogen	Labeling of pathogen for destruction and removal
T-cell receptor (T-cell membrane)	Proteins or certain lipids of pathogen	Induction of pathogen-specific humoral and cell-mediated immunity

RECEPTORS OF THE INNATE IMMUNE SYSTEM

Complement (bloodstream, tissue fluids)	Microbial cell-wall components	Complement activation, opsonization
Mannose-binding lectin (MBL) (bloodstream, tissue fluids)	Mannose-containing microbial carbohydrates (cell walls)	Complement activation, opsonization
C-reactive protein (CRP) (bloodstream, tissue fluids)	Phosphatidylcholine (microbial membranes)	Complement activation, opsonization
LPS-binding protein (LBP) (bloodstream, tissue fluids)	Bacterial lipopolysaccharide (LPS)	Delivery to cell-membrane LPS receptor (TLR-CD14-MD-2 complex*)
TLR2 (cell membrane)	Cell-wall components of gram-positive bacteria, LPS*. Yeast cell-wall component (zymosan)	Attracts phagocytes, activates macrophages, dendritic cells. Induces secretion of several cytokines
TLR3 (cell membrane)	Double-stranded RNA (dsRNA) (replication of many RNA viruses)	Induces production of interferon, an antiviral cytokine
TLR4 (cell membrane)	LPS*	Attracts phagocytes, activates macrophages, dendritic cells. Induces secretion of several cytokines
TLR5 (cell membrane)	Flagellin (flagella of gram-positive and gram-negative bacteria)	Attracts phagocytes, activates macrophages, dendritic cells. Induces secretion of several cytokines
TLR9 (cell membrane)	CpG	Attracts phagocytes, macrophages, dendritic cells. Induces secretion of several cytokines
Scavenger receptors (many) (cell membrane)	Many targets; gram-positive and gram-negative bacteria, apoptotic host cells	Induces phagocytosis or endocytosis

*LPS is bound at the cell membrane by a complex of proteins that includes CD14, MD-2, and a TLR (usually TLR4).

CLINICAL FOCUS

Drug Allergies—When Medicines Become Immunogens

Since World War II, penicillin has been used to successfully treat a wide variety of bacterial infections. However, the penicillin family of antibiotics is not without drawbacks. One is the role of penicillins and other antibiotics in the evolution of antibiotic-resistant bacterial strains. Another is their capacity to induce allergic reactions in some patients. Penicillin and its relatives are responsible for most of the recorded allergic reactions to drugs and 97% of the deaths caused each year by drug allergies.

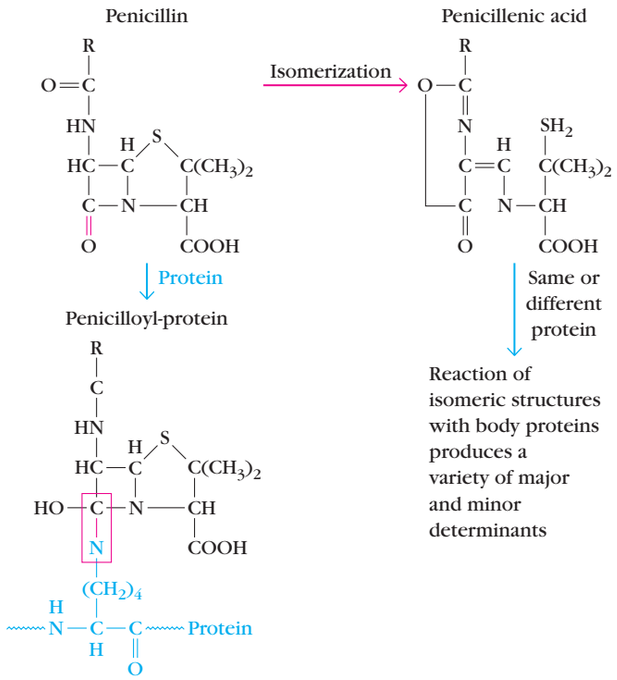
Allergies to penicillin and other drugs can be induced by small doses and are not consequences of the pharmacological or physiological effects of the drugs. An allergic response usually occurs about a week or so after the patient's first exposure to the agent, with typically mild symptoms often including hives, fever, swelling of lymph nodes, and occasionally an arthritis-like discomfort. Subsequent treatments with the drug usually cause much more rapid and often more severe reactions. Within minutes the throat and eyelids may swell. Grave danger arises if these symptoms progress to anaphylaxis, a physiological collapse that often involves the respiratory, circulatory, and digestive systems. Hives, vomiting, abdominal pain, and diarrhea may be a preamble to respiratory and circulatory problems that are life threatening. Wheezing and shortness of breath may be accompanied by swelling of the larynx and epiglottis that can block airflow, and a profound drop in blood pressure causes shock, frequently accompanied by weakened heart contractions.

The treatment of choice for anaphylaxis is injection of the drug epinephrine (adrenaline), which can reverse the body's slide into deep anaphylaxis by raising blood pressure, easing constriction of the air passages, and inhibiting the release from mast cells and basophils of the agents that induce anaphylaxis. Other drugs may be used to raise the low blood pressure, strengthen heart contractions, and expand the blocked airways. After a case of drug-induced anaphylaxis, affected individuals are advised to carry a notice warning future healthcare providers of the drug allergy.

Most drugs, including penicillin, are low-molecular-weight compounds that cannot induce immune responses unless they are conjugated with a larger molecule. Intensive investigation of allergy to penicillin has provided critical insight into the basis of allergic reactions to this and other drugs. As shown in the accompanying figure, penicillin can react with proteins to form a penicilloyl-protein derivative. The penicilloyl-protein behaves as a hapten-carrier conjugate, with the penicilloyl group acting as a haptenic epitope. This epitope is readily recognized by the immune system, and antibodies are produced against it. Some individuals respond to penicillin by producing significant amounts of a type of antibody known as immunoglobulin E (IgE). Once generated, these IgE antibodies are dispersed throughout the body and are bound by IgE receptors on the surfaces of mast cells and basophils,

TLR signaling can also result in the recruitment and activation of macrophages, NK cells, and dendritic cells, key agents in the presentation of antigen to T cells. The links to T cells and cytokine release shows the intimate relationship between innate and adaptive responses.

A search of the human genome has uncovered 10 TLRs, and the functions of six members of this PRR family have been determined. TLR2, often with the collaboration of TLR6, binds a wide variety of molecular classes found in microbes, including peptidoglycans, zymosans, and bacterial lipopeptides. TLR4 is the key receptor for most bacterial lipopolysaccharides, although TLR2 also binds some varieties of LPS. The binding of LPS by either of these TLRs is complex and involves the participation of three additional proteins, one of which is the lipopolysaccharide-binding protein mentioned above, abbreviated LBP. The first step in the process is the binding of LPS by circulating LBP, which then delivers it to a complex of TLR4 (or TLR2) with two additional proteins, CD14 and MD2. The engagement of LPS by this complex causes its TLR component to initiate a signal-transduction process that can produce a cellular response. Another family member, TLR5, recognizes flagellin, the major structural component of bacterial flagella. TLR3 recognizes the double-stranded RNA (dsRNA) that appears after infection by RNA viruses. As shown in Table 3-7, dsRNA is also recognized by dsRNA-activated kinase. Finally, TLR9 recognizes and initiates a response to CpG (unmethylated cytosine linked to guanine) sequences. These sequences are represented in abundance in microbial sequences but are much less common in mammalian sequences. Table 3-7 summarizes the receptors of adaptive immunity and lists many pattern-recognition receptors of innate immunity. The microbial targets and physiological sites of many PRRs are shown in Figure 3-11.



When nucleophiles such as amino groups or hydroxyl groups are present on soluble proteins or on the membrane of cells, they can react with penicillin and its relatives to form covalent linkages between host macromolecular structures and the drug. This is illustrated by the reaction of the free amino group of a lysine residue with penicillin (or with its spontaneously forming isomeric compounds, such as penicillenic acid) to produce protein-drug or cell-surface–drug derivatives. Such adducts are the major immunogenic species that elicit immune responses to this antibiotic. However, as indicated, other hapten-carrier conjugates of somewhat different structure are also formed and, because of their structural similarity, can also induce immune responses to penicillin. *[Adapted from N. F. Adkinson, 1995, in* Manual of Clinical Laboratory Immunology, *N. Rose et al., eds., American Society for Microbiology, Washington, D.C.]*

where they can remain for a long time. If a person with penicillin-specific IgE antibody bound to mast cells is subsequently treated with penicillin, there may be an allergic reaction. In fact, between 1 and 5 percent of people treated with penicillin develop some degree of allergy to it.

Penicillin is not the only drug against which patients can develop allergies. Others include streptomycin, aspirin, the so-called "sulfa-drugs" such as the sulfonamides, some anesthetics (e.g., succinyl choline), and some opiates. All of these small molecules first react with proteins to form drug-protein derivatives. When this happens, there is a possibility that the immune system will produce an anti-hapten response to the drug, just as with penicillin. Drugs (and their metabolites) that are incapable of forming drug-protein conjugates rarely elicit allergic reactions.

SUMMARY

- All immunogens are antigens but not all antigens are immunogens.
- Immunogenicity is determined by many factors including foreignness, molecular size, chemical composition, complexity, dose, susceptibility to antigen processing and presentation, the genotype of the recipient animal (in particular, its MHC genes), route of administration, and adjuvants.
- The sizes of B-cell epitopes range widely. Some are quite small (e.g., small peptides or small organic molecules), and are often bound in narrow grooves or deep pockets of the antibody. Protein B-cell epitopes are much larger and interact with a larger, flatter complementary surface on the antibody molecule.
- T-cell epitopes are generated by antigen processing, which fragments protein into small peptides that combine with class I or class II MHC molecules to form peptide-MHC complexes that are displayed on the surface of cells. T-cell activation requires the formation of a ternary complex between a T cell's TCR and peptide-MHC on antigen-presenting or altered self cells.
- Haptens are small molecules that can bind to antibodies but cannot by themselves induce an immune response. However, the conjugate formed by coupling a hapten to a large carrier protein is immunogenic and elicits production of anti-hapten antibodies when injected into an animal. Such injections also produce anti-carrier and anti-hapten/carrier antibodies as well.
- In the body, the formation of hapten-carrier conjugates is the basis of allergic responses to drugs such as penicillin.

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Review and quiz of key terms

- The innate immune system uses pattern-recognition receptors to recognize and respond to broad structural motifs that are highly conserved within microbial species but are generally absent from the host.

References

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USEFUL WEB SITES

http://www.umass.edu/microbio/rasmol/

RASMOL is free software for visualizing molecular structures that can be run on Windows-based, Macintosh, or Unix PCs. With it one can view three-dimensional structures of many types of molecules, including proteins and nucleic acids.

http://www.expasy.ch/

This is the excellent and comprehensive Swiss Institute of Bioinformatics (SIB) Web site, which contains extensive information on protein structure. From it one can obtain protein sequences and three-dimensional structures of proteins, as well as the versatile Swiss-PdbViewer software, which has several advanced capabilities not found in RASMOL.

Study Questions

CLINICAL FOCUS QUESTION Consider the following situations and provide a likely diagnosis or appropriate response.

a. Six hours after receiving a dose of penicillin, a young child who has never been treated with penicillin develops a case of hives and diarrhea. The parents report the illness and ask if it might be an allergic reaction to penicillin.
b. A patient who has never taken sulfonamides but is known to be highly allergic to penicillin develops a bladder infection that is best treated with a "sulfa" drug. The patient wonders if "sulfa" drugs should be avoided.
c. A student who is unaware that he had developed a significant allergy to penicillin received an injection of the antibiotic and within minutes experienced severe respiratory distress and a drop in blood pressure. An alert intern administered epinephrine and the patient's condition improved quickly. Frightened but impressed by the effectiveness of the treatment, he asked the intern why the shot of adrenaline made him feel better.
d. A pet owner asks whether the same mechanism that causes his allergy to penicillin could also be responsible for his dog's development of a similar allergy to the drug. (Please go beyond yes or no.)

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

a. Most antigens induce a response from more than one clone.
b. A large protein antigen generally can combine with many different antibody molecules.
c. A hapten can stimulate antibody formation but cannot combine with antibody molecules.
d. MHC genes play a major role in determining the degree of immune responsiveness to an antigen.
e. T-cell epitopes tend to be accessible amino acid residues that can combine with the T-cell receptor.
f. Many B-cell epitopes are nonsequential amino acids brought together by the tertiary conformation of a protein antigen.
g. Both T_H and T_C cells recognize antigen that has been processed and presented with an MHC molecule.
h. Each MHC molecule binds a unique peptide.
i. All antigens are also immunogens.
j. Antibodies can bind hydrophilic or hydrophobic compounds, but T-cell receptors can only bind peptide-MHC complexes.

2. What would be the likely outcome of each of the developments indicated below. Please be as specific as you can.

a. An individual is born with a mutation in C-reactive protein that enables it to recognize phospholipids in both bacterial and mammalian cell membranes.
b. A group of mice in which the CD1 family has been "knocked out" are immunized with *Mycobacterium tuberculosis.* Spleen cells from these mice are isolated and divided into two batches. One batch is treated with a lipid extract of the bacteria and a second batch is treated with a protein derived from the bacteria known as purified protein derivative (PPD).

3. Two vaccines are described below. Would you expect either or both of them to activate T_C cells? Explain your answer.

a. A UV-inactivated ("killed") viral preparation that has retained its antigenic properties but cannot replicate.

b. An attenuated viral preparation that has low virulence but can still replicate within host cells.

4. For each pair of antigens listed below, indicate which is likely to be more immunogenic. Explain your answer.

a. Native bovine serum albumin (BSA)
   Heat-denatured BSA
b. Hen egg-white lysozyme (HEL)
   Hen collagen
c. A protein with a molecular weight of 30,000
   A protein with a molecular weight of 150,000
d. BSA in Freund's complete adjuvant
   BSA in Freund's incomplete adjuvant

5. Indicate which of the following statements regarding haptens and carriers are true.

a. Haptens are large protein molecules such as BSA.
b. When a hapten-carrier complex containing multiple hapten molecules is injected into an animal, most of the induced antibodies are specific for the hapten.
c. Carriers are needed only if one wants to elicit a cell-mediated response.
d. It is necessary to immunize with a hapten-carrier complex in order to obtain antibodies directed against the hapten.
e. Carriers include small molecules such as dinitrophenol and penicillenic acid (derived from penicillin).

6. For each of the following statements, indicate whether it is true only of B-cell epitopes (B), only of T-cell epitopes (T), or both types of epitopes (BT) within a large antigen.

a. They almost always consist of a linear sequence of amino acid residues.
b. They generally are located in the interior of a protein antigen.
c. They generally are located on the surface of a protein antigen.
d. They lose their immunogenicity when a protein antigen is denatured by heat.
e. Immunodominant epitopes are determined in part by the MHC molecules expressed by an individual.
f. They generally arise from proteins.
g. Multiple different epitopes may occur in the same antigen.
h. Their immunogenicity may depend on the three-dimensional structure of the antigen.
i. The immune response to them may be enhanced by coadministration of Freund's complete adjuvant.