CHAPTER 7

Host Immune Response to Viral Infection: The Nature of the Vertebrate Immune Response

The human lymphatic system shown in Fig. 7.1 is part of the general circulatory system and provides the most profound response to the presence of foreign proteins in the body. When any protein that is not part of the vast protein repertoire making up the vertebrate host is presented to the immune system by an **antigen-presenting cell (APC)**, both B cell immunity (**humoral immunity**) and T cell immunity (**cell-mediated immunity** (CMI)) are mobilized. Such a foreign protein is usually termed an *antigen* and can be derived from an invading pathogen like a virus (bacterial or metazoan), or it can be a novel cellular protein expressed as a result of abnormal growth properties of the cells—a **tumor antigen**. An antigen can also be all or part of a perfectly normal protein from an animal, plant, or microbial source to which the particular animal in which it is presented has never been exposed.

Lymphatic cells are produced, differentiate, and mature in certain specialized tissues, including bone marrow, spleen, and thymus. They circulate throughout the body in the lymphatic system and can migrate between cellular junctions into tissue. They are most concentrated in **lymph nodes** where stimulation to provide a **systemic immune response** can begin. B cells produce **antibodies** that are secreted proteins able to combine specifically with the antigenic determinants on proteins. Immune T cells have antigen-binding sites on their surfaces and upon encountering

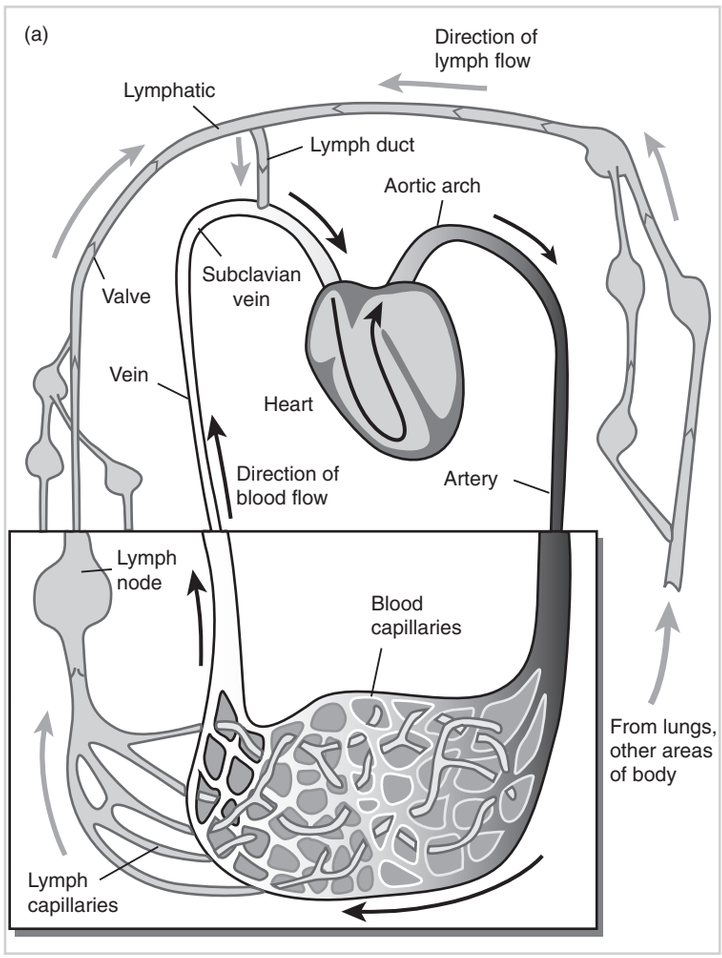

Fig. 7.1 The human lymphatic system. The lymphatic system is the principal organ of the immune system. *a.* The relationship between the lymphatic circulation and that of the blood. *b.* Some of the important components of the lymphatic system as related to the immune response.

antigen-bearing cells, interact with them, resulting in lysis of the antigen-presenting cells. They also function in the development of B cell immunity.

Together, these two arms of the immune system interact to allow the host to detect and destroy or render noninfectious (inactivate or neutralize) both free virus and virus-infected cells that display viral proteins at their surface. A general outline of the interaction between an antigenic pathogen and the immune system is shown in Fig. 7.2.

The immunological structure of a protein

In any protein, certain clusters of amino acids (usually between 10 and 12) are able to interact with the appropriate antigen-recognizing T cells or antibody-producing B cells to lead to proliferation of those cells. These clusters are called antigenic determinants (**epitopes**). B cell reactive epitopes are usually **hydrophilic**, and thus, hydrated. A viral protein can have none, a few, or many antigenic determinants, depending on its protein structure, amino acid sequence, sequence relation to cellular proteins, and other factors. Two proteins can share some of the same or closely related determinants, and the closer the relation between the proteins, the greater the shared ones. This is why closely related viral serotypes share a high degree of immunological reactivity. A schematic representation of epitope types present in proteins is shown in Fig. 7.3.

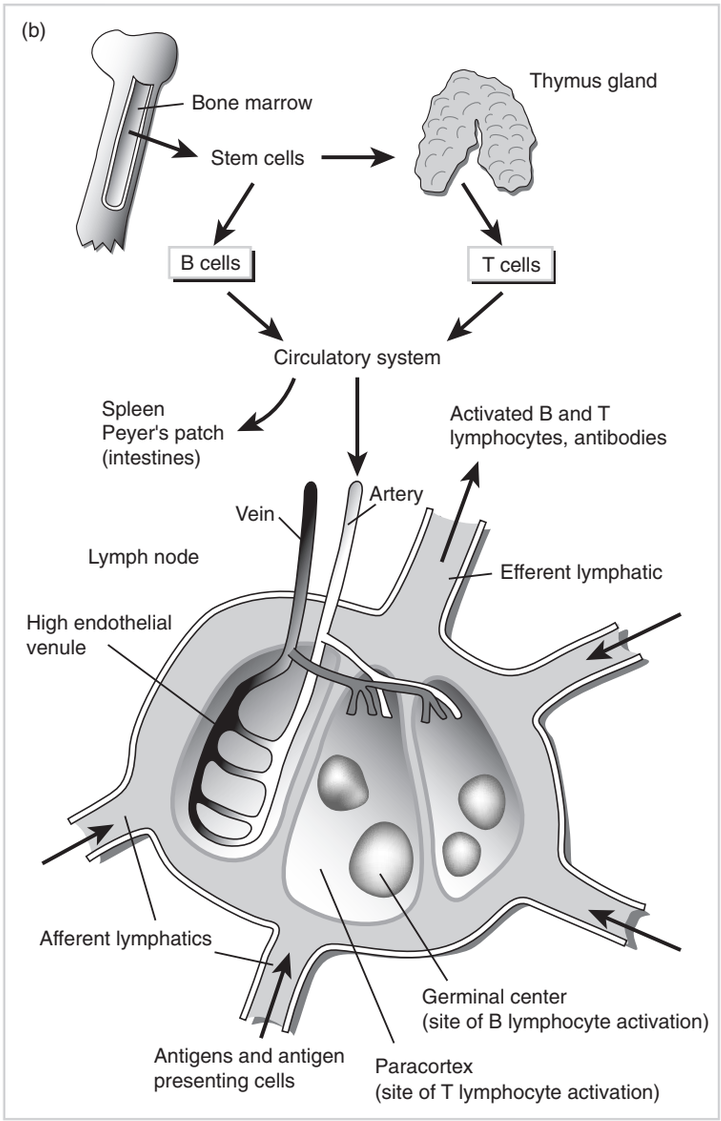

Fig. 7.1 *Continued*

Epitopes are often composed of a specific sequence of amino acids. With such an epitope, denaturation of the antigenic protein will have no effect on its properties or how it is presented to the immune system. Such determinants expressed in a protein in either its native or denatured state are called *sequential epitopes*.

Epitopes can also be sensitive to the structure of the protein region where they occur. For example, they could be made of amino acids that have been brought near each other by protein folding or conformation. These are **conformational epitopes**; such will be sensitive to denaturation (disruption) of the protein structure.

Either sequential or conformational epitopes can be in the interior of a protein where they are not normally "seen" by the humoral immune system. These are *buried determinants*. Many of these are sequential and can be exposed by denaturation of the protein. A buried conformational determinant could be exposed by proper limited degradation of the protein, or by denaturation of the protein followed by its being refolded in a form that exposed the epitope.

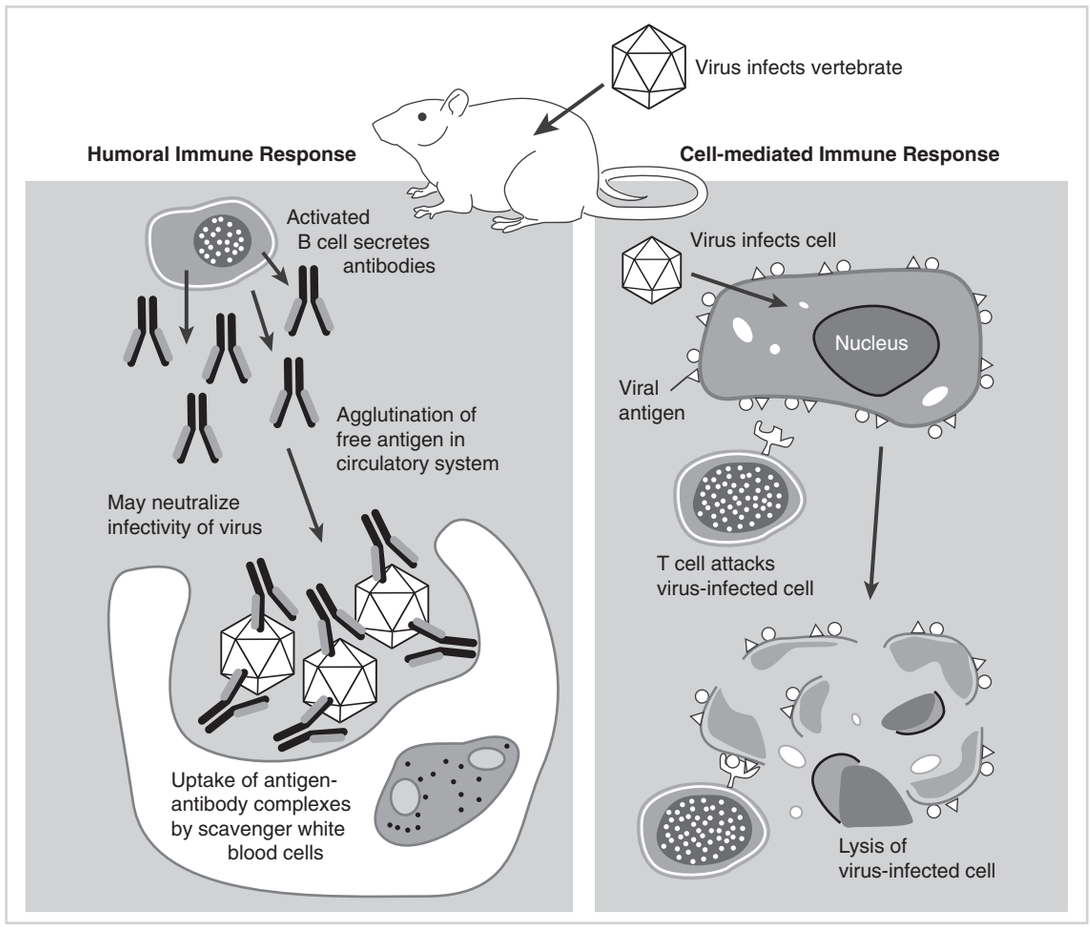

Fig. 7.2 T and B cells in immunity. T lymphocytes play the central coordinating role in evoking the immune response. Upon activation by interaction with a specific antigenic determinant with which they can interact, they proliferate and carry out the functions shown. B cells reactive with specific antigens require reactive T cells for their maturation. Upon maturation, they secrete antibody proteins that bind to antigenic determinants.

PRESENTATION OF VIRAL ANTIGENS TO IMMUNE REACTIVE CELLS

Local versus systemic immunity

The response to virus infection, including the immune response, begins as soon as viruses enter. The cell-based interferon defense is an important factor in local immunity, and is described in some detail in Chapter 8. Further, as soon as a cell of the immune system contacts a foreign protein presented to it in the proper cellular context, the processes involved in acquired immunity can commence. Since cells of the immune system are present throughout the body, some immune reactions occur as soon as a virus initiates an infection at its point of entry. This immune response is necessarily limited to the few immune cells initially present. This is especially true in an immunologically naive host (i.e., one that has never encountered the specific antigen before). Despite this constraint, which will be increasingly nullified by the proliferation of immune reactive cells resulting from systemic immunity (see below), local immunity is very important in limiting the ability of

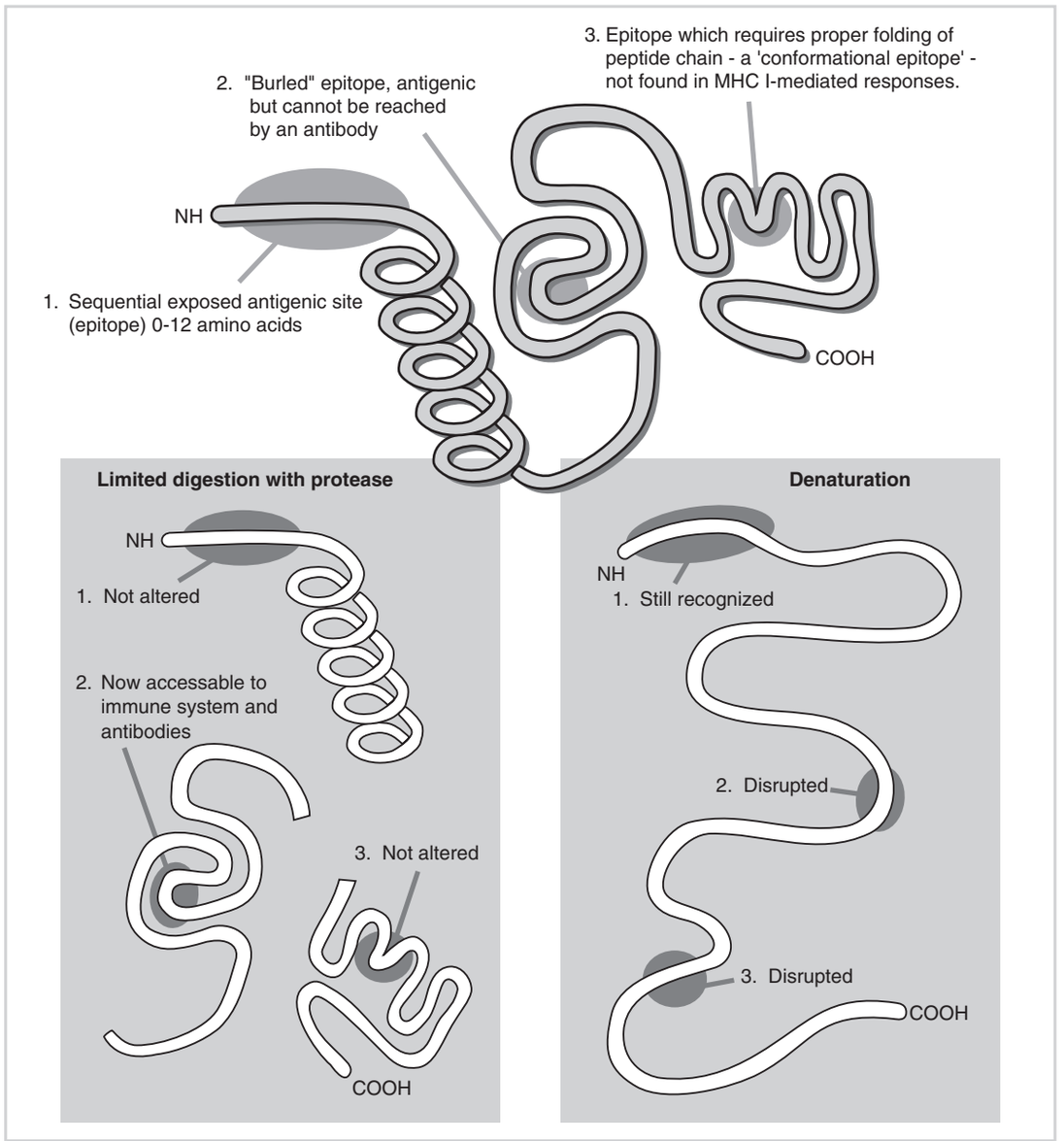

Fig. 7.3 The antigenic structure of a protein. Specific groups of amino acids (usually hydrated) serve as specific antigenic determinants, or epitopes in an antigenic protein. Some of these are insensitive to the protein's physical structure; others require a specific conformation for presentation.

small amounts of infectious virus to become established and spread. Further, local immunity is the "first line of defense," providing an effective barrier to reinfection of the immune host.

Systemic immunity refers to the more generalized immune response engendered when a pathogen breaches the primary site of infection or replicates to a high level in the host. This immunity involves the entire immune system and the generation and mobilization of large numbers of immune reactive cells in lymphatic tissue. While this response is a very strong one, its onset is delayed until the immunogenic pathogen establishes itself in high-enough numbers to trigger it. The process is delayed further because of the time needed for immune cell replication and the

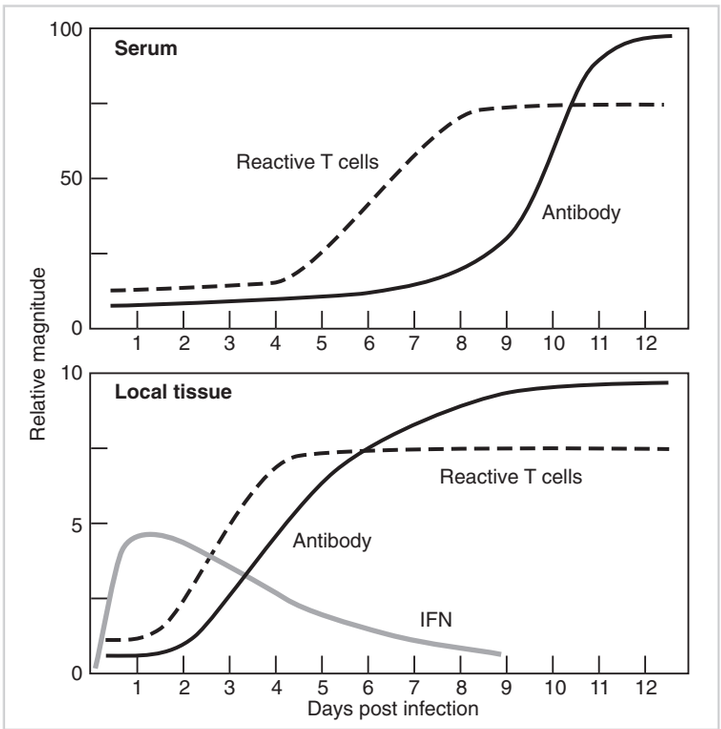

Fig. 7.4 Schematic representation showing differences in the intensity and time of appearance of local versus systemic immunity against a typical virus infection in mice. (IFN, interferon, Figure courtesy of D.C. Bloom.)

differentiation linked to this process. Figure 7.4 is a schematic representation of differences in intensity and kinetics of local versus systemic immunity.

Role of the antigen-presenting cell in initiation of the immune response

Any protein and many other macromolecules can be antigenic, but antigens must be "processed" and then presented at the surface of the cell bearing them (**antigen presenting cell**) in the proper context to be able to evoke an immune response. This context is as a complex with one of two closely related cell surface glycoproteins, the **major histocompatibility** proteins. The MHC determinants ensure that only macrophages from the same organism can present antigens to the immune system. There are two basic pathways through which cells present antigens. The first, which is a function of nearly all cells, is the presentation of endogenously expressed antigens on the surface via the type I **major histocompatibility complex** (MHCI). As proteins are being synthesized portions are complexed with a group of cellular proteins named **ubiquitins**, which target the proteins to proteolytic vesicles (**proteasomes**) where they are partially degraded into epitope-sized peptides. These peptides are then moved via transporter proteins (**TAP**s) into the Golgi apparatus where the peptides associate with newly synthesized MHCI glycoproteins and are presented on the surface of the cell (see Fig. 7.5a). These MHCI complexes serve as targets for surveying $CD8^+$ T cells, and if reactive, the cells bearing the antigen are destroyed. In this way, the immune system surveys all cells for the synthesis of foreign or abnormal proteins. This endogenous antigen presentation is important in the early immune detection of viral-infected cells, and is clearly a major factor in local immunity.

The establishment of systemic immunity and immune memory require a relatively large population of freely circulating, relatively short-lived effector T cells that can recognize the antigen in

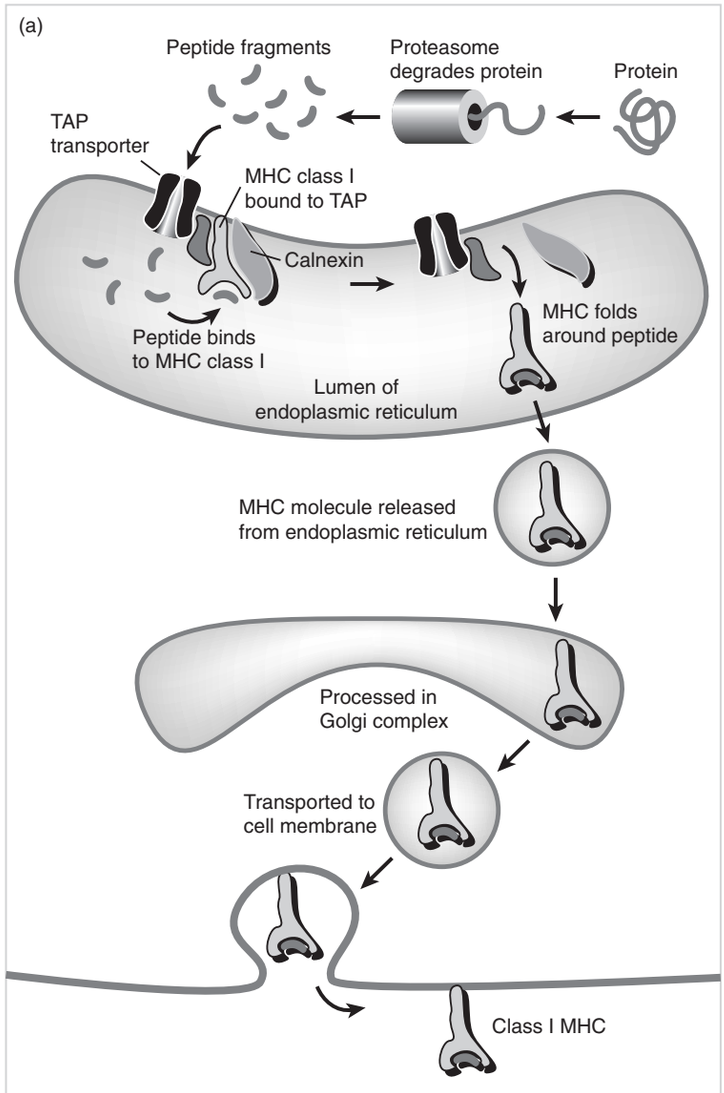

Fig. 7.5 The processing of a foreign antigen and stimulation of the immune response. As described in the text, an antigenic protein can only stimulate the immune response when it is processed by a macrophage and then presented to cells of the immune system in lymph nodes in the presence of histocompatibility antigens. The processing is relatively rapid and involves partial degradation of the antigenic protein and expression of antigenic portions on the surface of the antigen-presenting cell. (ER, endoplasmic reticulum.) *a.* MHCI antigen processing and presentation. *b.* MHCII processing and presentation.

question. This primarily occurs via the activity of long-lived specialized **dendritic cells** that were formed in the bone marrow and migrate to the epithelium where they remain. These and certain other cells of the immune system are often termed "professional antigen presenting cells", because of this primary role in evoking systemic immunity. Antigenic proteins or complexes are recognized in manners that are not fully understood, and are internalized and partially digested by receptor-mediated endocytosis. Fragments of antigens containing epitopes are re-expressed on the cell surface in the presence of cellular type II major histocompatibility complex (**MHCII**) proteins. The antigenic fragment and the major histocompatibility complex (MHC) molecules together form a surface structure that can be recognized by certain B and $CD4^+$ T cells in lymph nodes to begin the amplification of cells able to recognize the antigen—this is shown schematically in Fig. 7.5b. MHCII-mediated antigen presentation occurs in lymph nodes. Because antigen concentration must reach a high-enough level to evoke the immune response, the process takes time and occurs only following a lag after initial infection and early replication of the virus. This delay is important in virus infections—such as HSV infections—where virus can invade sensory neurons and estab-

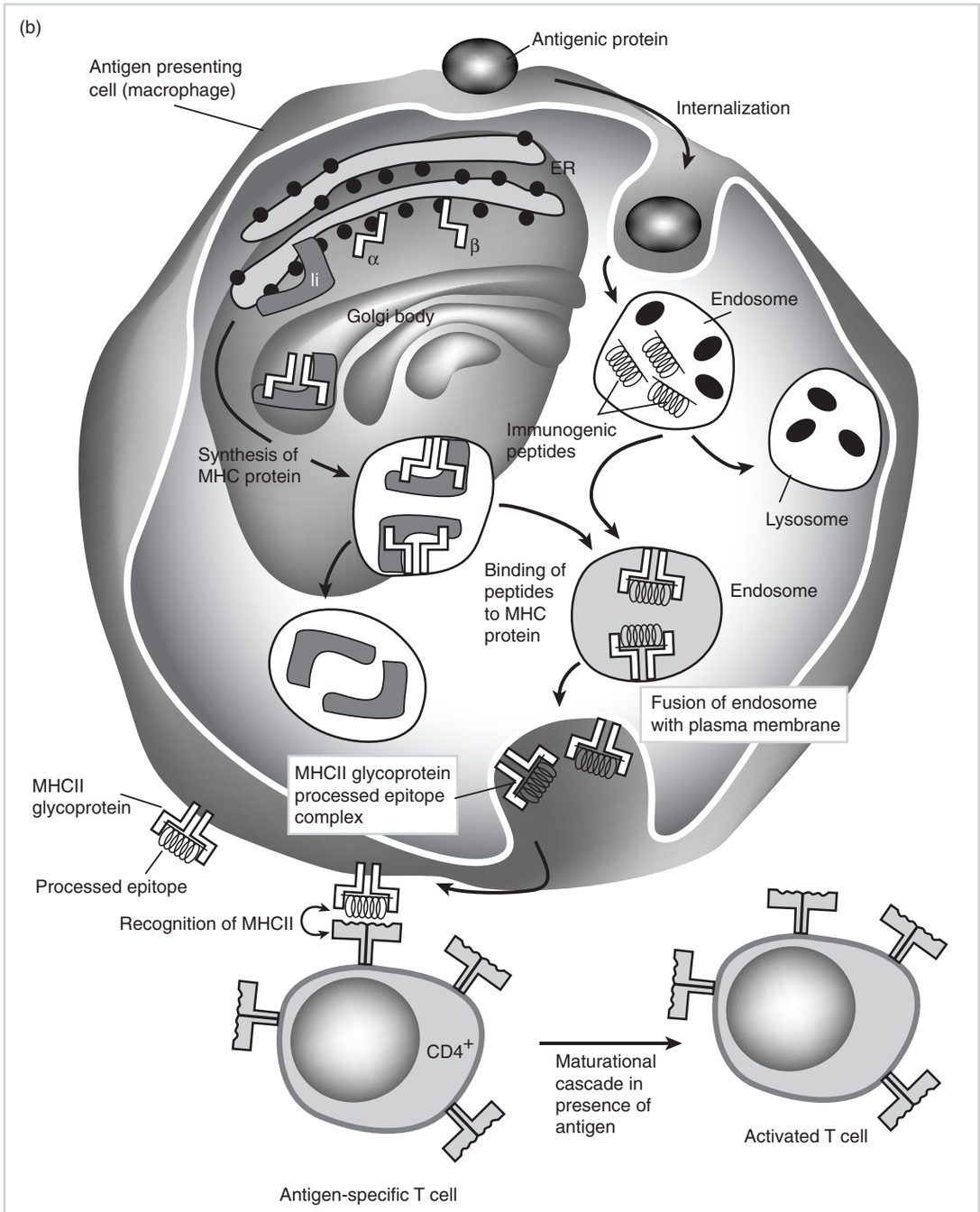

Fig. 7.5 *Continued*

lish latent infections before a powerful immune response is achieved. Indeed, HSV, like some other viruses, can actually interfere with the MHCI-mediated early presentation of its antigenic proteins at the surface of the infected cell by the action of a specific viral protein expressed immediately following infection.

Some viruses (notably HIV) can survive internalization by macrophages, and their presentation to T cells leads to infection of lymphocytes. HIV can replicate in lymphocytes, and eventually replication of the virus in infected lymphatic cells leads to destruction of the immune system.

As the T and B cells able to interact with the presented epitope continue to proliferate, immature B cells with surface receptors that can bind to antigen also internalize and process the antigen. These B cells provide an alternative mechanism for presenting antigen in the lymph nodes.

The internalization and processing of antigens is clearly of paramount importance to the ability to generate effective immunity. In addition to the generation of sequential determinants, the host can generate immune responses to complex conformational epitopes, such as portions of dimeric and multimeric proteins found at the surface of the virus. Indeed, the host preferentially mounts strong antigenic responses to the surface proteins of virus. Part of the reason for these responses is that such proteins are present in large amounts and are at the "interface" between the infection and the host antigenic response. Other factors are also involved, including structural features of the proteins, inherent resistance to extensive degradation by surface proteins, and numerous poorly characterized portions of the antigen-presenting pathway.

Clonal selection of immune reactive lymphocytes

When antigens are presented to immune cells that can recognize them, those T and B cells are stimulated to proliferate. As shown in Fig. 7.6, the process of **clonal selection** takes place because each specific antibody-producing B cell and each specific epitope-recognizing T cell are derived from a single reactive cell (i.e., clones of that cell). This process takes place mainly in the lymph nodes because of the high concentration of cell populations that must interact. The ability to generate clones of antibody-producing B cells in the laboratory has provided an extremely important tool for studying the functional structure and relationships between various cellular and viral proteins. Some basic techniques using such material are outlined in Chapter 12.

As they are stimulated by the presence of a specific epitope that they recognize, B cells divide and differentiate (mature). Fully differentiated B cells secrete soluble antibodies. One class of effector T cells (*helper* or T_H *cells*) mediates the maturation of B cells. Another class, the T_C cells, attack and destroy cells with foreign antigens on them, such as virus-infected cells. A third class of T cells (*suppressor* or T_S *cells*) suppress the immune response toward the end of the "crisis" when immunity is at a high level and antigen levels begin to decline.

Immune memory

The immune system "remembers" the antigenic response and can rapidly respond to reexposure to the antigen. Immune memory is mediated by long-lived "memory" T and B cells. Such memory cells reside mainly in lymph nodes. As antigen persists, the cells that respond to it continue to proliferate. While most have a finite lifetime and then undergo apoptosis, memory cells do not function in dealing with the antigen, but rather are long-lived and remain in lymph nodes. A second stimulation with the antigen results in rapid interaction of the antigen with such memory cells and a secondary (remembered) immune response that is more rapid and more extensive than the first or primary response. The effect of immune memory on the strength and speed of the immune response is shown in Fig. 7.7.

Complement-mediated cell lysis

Although T cells have a primary role in the destruction of cells bearing foreign antigens, B cells can also destroy antigen-bearing cells by use of the complement system, which leads to *complement-mediated cell lysis*. This system works because cells with antibodies bound to them trigger a cascade of

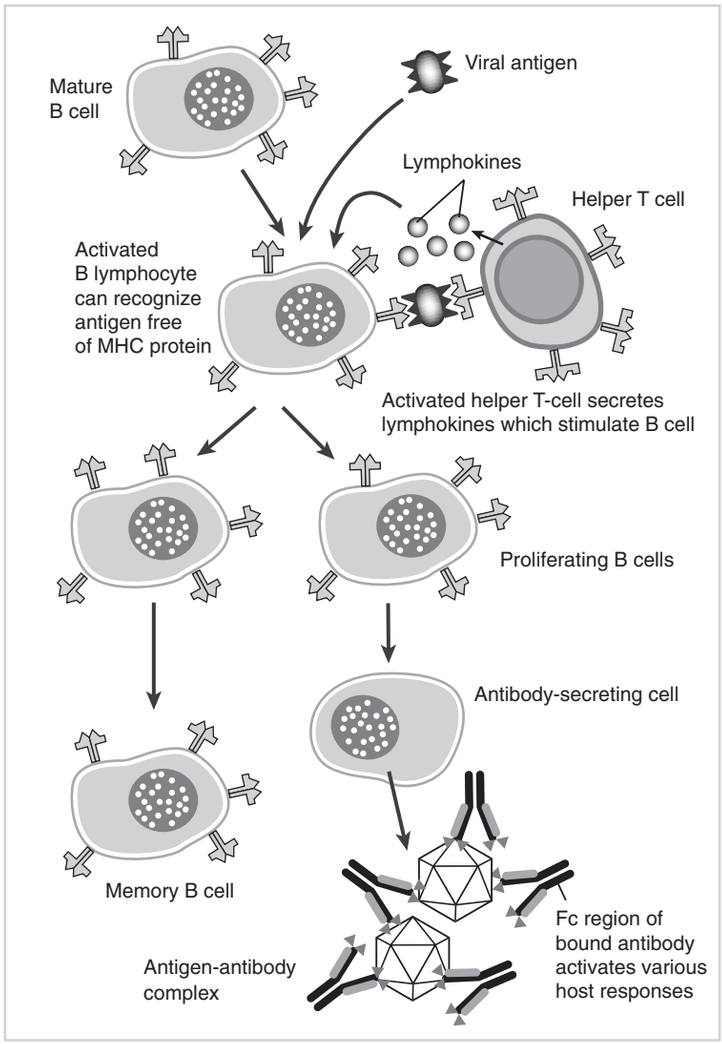

Fig. 7.6 The clonal selection of B lymphocytes. Only the B lymphocytes reactive with a specific epitope can be stimulated to mature by the action of a helper T lymphocyte. Specific mature B cells secrete specific types of antibody molecules, but the same epitope will result in only the stimulation and maturation of B-cell clones reactive with it.

interactions with serum complement proteins that leads to destruction of the cell; this process is outlined in Fig. 7.8.

CONTROL AND DYSFUNCTION OF IMMUNITY

The T and B cells with antigenic recognition sites having the highest affinity for a given epitope are stimulated most efficiently. As general levels of antigens fall late in infection and during recovery, lower levels of high-affinity antigens can continue to stimulate immunity. Thus, the nature of the immune response changes with time after infection. A recovering patient will generally have higher-affinity and more specific antibodies than will an individual early in the course of a disease.

Suppressor T cells mature very late in the immune response and shut down immunity. These cells are important to the regulation of immunity. If they do not function properly, hyperimmune responses such as allergic reactions may occur. If they function too well, inadequate immunity may result. HIV infections appear to destroy many effector T cells but not suppressor ones, so one of the

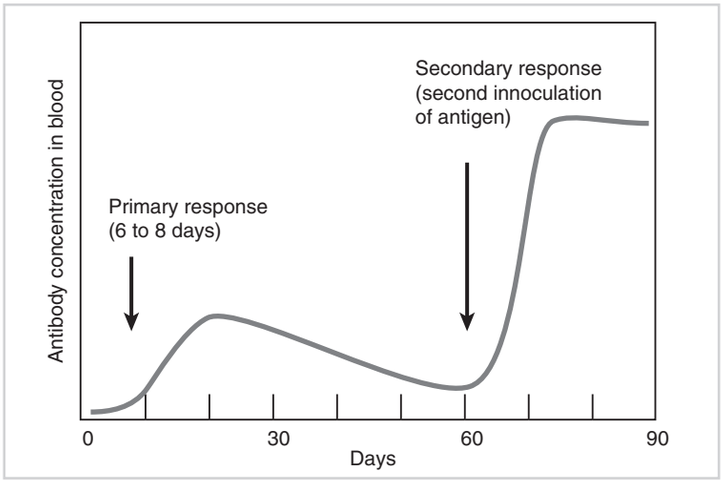

Fig. 7.7 Immune memory. The first exposure to an antigen results in the primary response, which occurs only after a week or so. During this time, maturation of immune-reactive cells is taking place. Once the primary response occurs, antibody and reactive T cells decline to a low level. Upon restimulation with the same antigen, the memory lymphocytes are rapidly mobilized and a more intense and more rapid immune response follows.

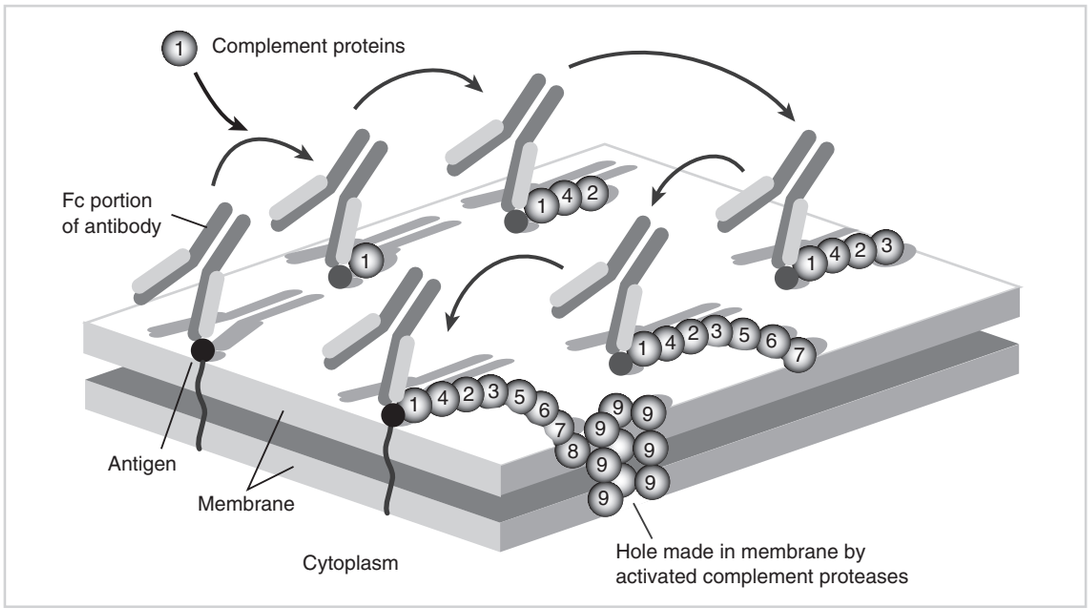

Fig. 7.8 The maturational cascade of serum complement proteins upon binding to an antigen-antibody complex on the surface of a cell. A portion of the antibody molecule that is not involved in binding to the epitope of the antigen specifically triggers this cascade.

problems in AIDS is that in addition to the destruction of immune cells, there is a deficit in the production of new ones.

Other types of immune pathologies include autoimmune diseases where the immune system destroys seemingly healthy tissue in the body. This can be due to the immune system attempting to destroy cells that express viral antigens but that are otherwise healthy.

An example of an autoimmune pathology due to viral infection and persistent presentation of antigen is subacute sclerosing panencephalitis (SSPE), which is a pathological response to persistence of measles virus antigen in neural tissue. This was briefly described in Chapter 4. Some other autoimmune diseases, such as multiple sclerosis, are thought to be caused by a previous virus infec-

tion and apparent recovery. It is suggested that a previous infection with a virus (perhaps years before) can lead to immune pathology—in this case demylenation of neurons. The exact mechanism of such pathology is not known, but a process termed "**molecular mimicry**" where a specific epitope of the pathogen bears similarity to one in the host tissue is suspected. Here, during the course of a normal immune response against the invading pathogen, normal tissue is also now recognized as foreign. This is known to be the mechanism for the role of group A Streptococcus in rheumatic fever where the robust immune response to the bacterial epitope leads to problems because of similarity to one in heart tissue. This mechanism has not been proved for multiple sclerosis, and indeed, such cases require very careful statistical evaluation of long-term medical records to demonstrate correlations.

Specific viral responses to host immunity

The immune response is an effective one, and plays a constant role in selection against viruses that do not mount an efficient infection. Despite effectiveness of the immune response, it is clear that many virus infections survive and thrive in the setting of the host's immune capacity. Indeed, the great majority of nuclear-replicating DNA viruses establish long-lasting associations with their hosts. Clearly they are able to deal with host attempts to clear the infection. A major factor in virus survival is the fact that viruses mount many effective counter responses to the immune response. Some of these are essentially passive while others involve virus-mediated blockage of specific portions of the immune response.

Passive evasion of immunity—antigenic drift

All animal viruses occur in antigenically distinct forms or serotypes. The number of forms varies with the type of virus. For example, there are three major serotypes of poliovirus, more than 40 for adenovirus, and as many as 100 for papillomaviruses. A serotype is stable and may be confined to a specific geographic location, and prior infection with one serotype of a specific virus will lead to no or only partial protection from reinfection with another.

Because RNA-directed RNA replication has no built-in enzymatic error-correction mechanism, in contrast to DNA replication, RNA viruses are generally more susceptible to the generation of mutations leading to serotype formation than are DNA viruses. This process is often termed **antigenic drift**, and such drift is probably responsible for the large number of serotypes of rhinoviruses (more than 100), and is clearly responsible for the drift in influenza virus serotypes.

This mechanism for drift is countered by other factors that tend to favor antigenic "conservatism." For example, many RNA viruses (e.g., poliovirus) do not exhibit large numbers of serotypes, and even where there is extensive drift, as with influenza, the internal proteins are antigenically stable.

One factor in stabilizing protein sequences even when they are encoded by highly mutable RNA sequences is that important functional constraints on the amino acid sequence of viral proteins have enzymatic or precise structural functions. Such constraints do not operate with the same lack of tolerance for variation in the glycoproteins of enveloped viruses.

Passive evasion of immunity—internal sanctuaries for infectious virus

Some viruses can evade the immune response of the host by establishing persistent or latent infections in tissue that is not subject to extensive immune surveillance. A classic example is the ability of HSV to establish latent infection in nondividing sensory neurons. Another example is the ability of respiratory syncytial virus to replicate at low levels in the mucous membranes of the nasopharynx where secretory antibodies provide protection against invasion by the virus, but

cannot clear it. The highly localized replication of papillomaviruses, such as those causing skin warts, is another example of virus infection in a localized area that is far removed from intense immune surveillance.

Passive evasion of immunity—immune tolerance

The immune system of fetuses and neonates is immature. This is an important strategy in the survival of the fetus as it develops in an antigenically distinct individual: its mother. Fetal and neonatal infections with viruses that cause generally mild infections in an immune-competent individual can be devastating. Rubella causes severe developmental abnormalities of the nervous system when it infects a developing fetus, and the fact that the virus does not evoke lasting immunity in adults means that it is a threat even to a mother who has been infected previously. A primary or reactivating HSV infection of the mother at the time of birth can lead to neonatal encephalitis with grave prognosis, and neonatal and uterine infections with cytomegalovirus are strongly linked to neurologically based developmental disorders.

At least one group of viruses, the arenaviruses, utilizes the ability to selectively accommodate themselves to the developing immunity of the neonate. These viruses, of which lymphocytic choriomeningitis virus (LCMV) is the best-characterized laboratory model, persist in populations of rodents and are transmitted to newborns from the infected mother. The mouse develops relatively normally with persistent viremia and shows an impaired immune response to LCMV. The tolerant mouse has circulating antibody that is reactive with the virus but cannot neutralize it. Further, there is a lack of T-cell responsiveness to the virus. If an immune-competent adult mouse is infected with LCMV, a robust immune response is mounted, but the infection is usually fatal!

The mechanism for establishing this immune tolerance is complex; it involves selection of specific viral genotypes with the ability to infect macrophages and some other cells of the immune system during the early stages of infection of the infant. This infection results in suppression of specific immunity against the virus.

Interestingly, the virus that is spread between individuals has tropism for neural tissue. These neurotropic and lymphotropic viruses differ only in a single amino acid in both the viral glycoprotein and the viral polymerase. The two variants are generated by random periodic mutations during replication of the resident virus in the animal, and while the neurotropic variant has little effect in the immune-tolerant animal, it causes severe disease in an uninfected adult. Similar patterns of infections are seen with other arenaviruses, several of which—including Lassa fever virus—are pathogenic for humans.

Active evasion of immunity—immunosuppression

Infections with a number of viruses lead to a transitory or permanent suppression of one or several branches of host immunity. Infectious mononucleosis caused by primary infection with EBV is a self-limiting generalized infection characterized by a relatively large induction of suppressor T lymphocytes. This not only results in the virus being able to maintain its infection effectively, but also results in the individual who has the infection being more susceptible to other infections. Some retroviruses, especially HIV, are able to specifically inhibit T cell proliferation by the expression of suppressor proteins. Further, the continued destruction of T lymphocytes by HIV replication eventually leads to profound loss of immune competence: AIDS.

The polydnaviruses of certain wasps illustrate an evolutionary adaptation between virus and host based on the virus's ability to actively suppress immunity. This virus (mentioned in Chapter 1) is maintained as a persistent genetic passenger in the ovaries and egg cells of parasitic wasps. These wasps lay eggs in caterpillars of another insect species, and the developing larvae feed on the caterpillar as they develop. The polydnavirus inserted into the caterpillar along with the wasp egg in-

duces a systemic, immunosuppressive infection so that the caterpillar cannot eliminate the embryonic tissue at an early stage of development. If wasps without such viruses inject eggs into the caterpillar host, there is a significant reduction in larval survival.

Active evasion of immunity—blockage of MHC antigen presentation

Both adenovirus and HSV specifically inhibit MHCI antigen presentation. In each case, a specific virus protein that mediates this blockage is expressed. While it is apparent that the slowly replicating adenovirus will greatly benefit from its ability to interfere with host immunity, it requires a moment of reflection to see the importance of the blockage of MHCI antigen presentation by HSV, which replicates very rapidly and efficiently in the cells it infects. Here, it is likely that the value is found in the earliest stages of reactivation from latent infection where small amounts of virus must be able to initiate infection in a host that has a powerful immune memory biased against HSV replication.

Consequences of immune suppression on virus infections

While some viruses are able to either mildly or profoundly suppress immunity during the course of infection and pathogenesis, immune suppression is also an important tool in certain medical conditions. Examples include the need to suppress host cell-mediated immunity prior to organ or tissue transplantation. Immune suppression also results from some types of intravenous drug abuse.

Major complications from such suppression are reactivating herpesvirus infections such as varicella zoster (chicken pox) and cytomegalovirus infection. Of course, the same problems can occur when the immune system is disrupted by viral infections such as with HIV. A potentially more critical complication of significant populations of individuals evidencing immune suppression results from their serving as potential selective reservoirs for the development of antigenic and drug-resistant strains of pathogens. For example, the current increase in appearance of antibiotic-resistant tuberculosis is linked definitively to a combination of incomplete drug therapy and HIV and drug-induced immunosuppression in critical urban and Third World populations.

MEASUREMENT OF THE IMMUNE REACTION

Measurement of cell-mediated (T cell) immunity

Cell-mediated immunity requires incubation of immune lymphocytes with a target (usually a cell) and then measurement of a specific T cell response. This can be difficult and tricky, but for measurement of T cell-mediated cell lysis, the release of radioactive chromium from target cells is a convenient method. Target cells are incubated under conditions such that they incorporate the radioactive metal. The cells are rinsed so that the only radioactivity is inside the cells. Thus, the radioactivity can sediment to the bottom of a centrifuge tube under low gravity force (low speeds). In the presence of reactive killer T cells, the target cells are lysed and the "hot" chromium enters the solution and cannot be sedimented under low speeds.

A numerical assessment of the number of reactive lymphocytes can also be carried out by measuring cell replication as a response to a specific antigen. Circulating white blood cells are incubated with antigen and a radioactive nucleoside precursor to DNA. As T lymphocytes proliferate in response to antigen, they will incorporate this radioactive precursor. A measure of the incorporation of radioactivity in comparison to a control culture can be made and expressed as a lymphocyte **stimulation index**.

Another method for measuring T-cell immunity is to incubate antigen-bearing cells with lym-

phocytes. Reactive T lymphocytes will form *rosettes* around the antigen-bearing cell, and these can be observed and counted in the microscope.

Measurement of antiviral antibody

Antibody molecules are secreted glycoproteins that have the capacity to recognize and combine with specific portions of viral or other proteins foreign to the host. As described in Chapter 12, antibody molecules have a very specific structure in which the antigen-combining sites, which comprise variable amino acid sequences, are at one location on the antibody molecules while a region of fixed amino acid sequence is found at another location. This constant region (**Fc region**) has a major function in mediating secretion of the antibody molecule from the B lymphocyte expressing it. Another major function is to serve as a signal to cells and other specific cellular proteins that the molecule bound to the antigen is, indeed, an antibody.

Enzyme-linked immunosorbent assays (ELISAs)

A number of methods to measure antibody reactions involve use of the antibody molecule's Fc region as a "handle." Extremely sensitive methods known collectively as enzyme-linked immunosorbent assays **(ELISAs)** use enzymes that can process a colorless substrate into a colored product bound to the Fc region of an antibody molecule. When the antibody then is bound to an antigen, the enzyme affixed to the Fc region will also be bound. If the antigen-antibody complex is then incubated with appropriate substrates for the bound enzyme, the generation of color can be used as a measure of the antibody present. Examples of the method are outlined in Fig. 7.9.

ELISAs are of tremendous value for rapid diagnosis, and have great commercial significance. For example, if an antigenic peptide is bound to an insoluble matrix such as a flexible plastic strip onto which dry reagents are included and this strip is dipped into a plasma preparation that contains antibody against the peptide, a color will develop. Even if the amount of antibody is very low, incubation for a long-enough period will generate some color as long as the enzyme used is relatively stable. The method is quite adaptable to quantitative as well as qualitative analysis, and can be adapted for use with automated equipment. A number of kits are currently commercially available where a small sample of body fluid that might contain either a virus or an antibody of interest can be spotted and dried. The kit is then sent to a laboratory where it can be quantitatively analyzed.

The use of lasers and microtechnology developed in the electronics industry promises to provide even more revolutionary changes to our ability to detect extremely small amounts of viral antigens or antibodies in test material. A microchip can be synthesized with a huge number of different potential antigens bound to it, and this can be incubated with unknown antibody and then subjected to either an ELISA or another method to generate a fluorescent signal where an antigen-antibody complex is formed. This can be rapidly scanned with a laser beam and fluorescent microscope or alternatively, in a solid-state detection device. Such methods make it potentially possible to screen a given serum sample for the presence of all or nearly all identified pathogenic agents in a few hours!

Neutralization tests

Some ways to measure the reaction between specific antibody molecules and an antigen involve the loss of specific functions by the target virus. Many antibodies will block the ability of a virus to initiate an infection in a cultured cell, and thus block the formation of a center of infection or *virus plaque*. Plaque assays are described in Chapter 10, and the inhibition of plaque formation is termed an infectivity *neutralization* or *neutralization* of a virus. Here, a target virus with a known titer is incubated with test antibody dilutions. The more concentrated and specific the antibody, the more

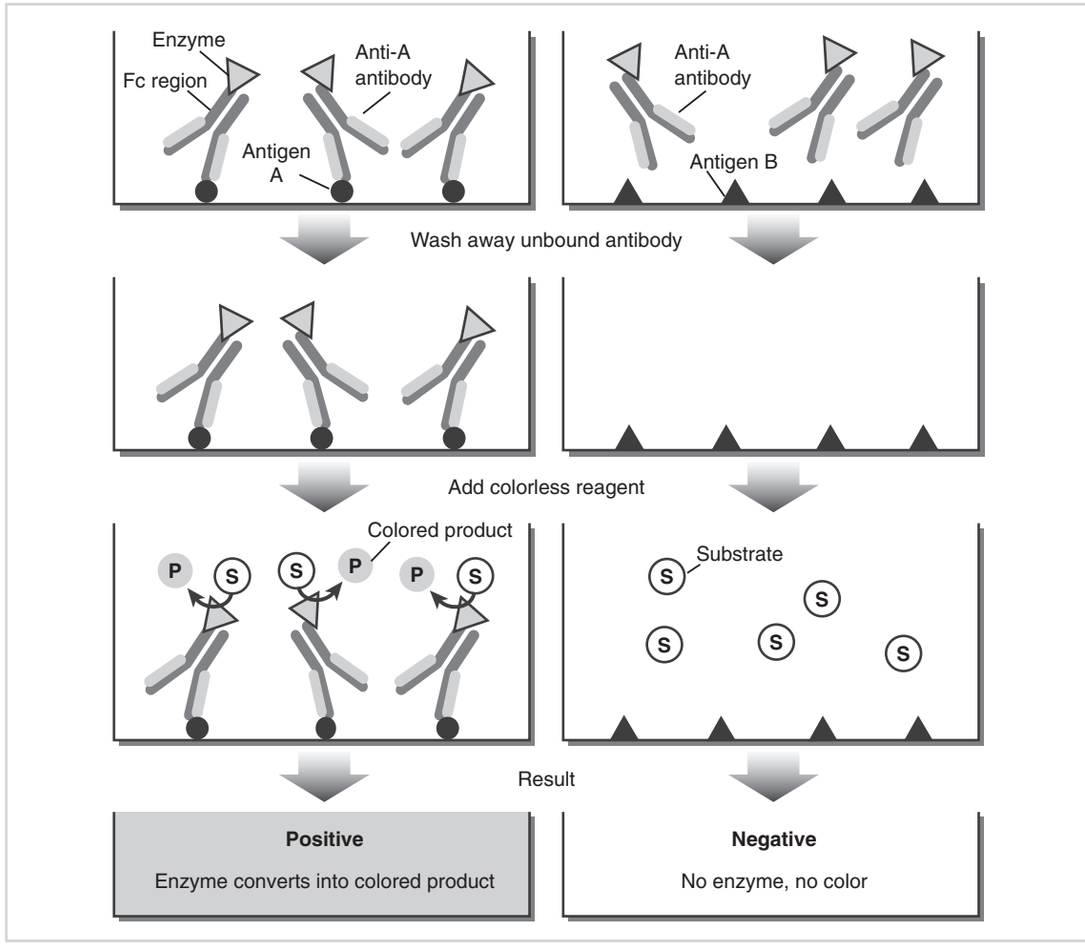

Fig. 7.9 An enzyme-linked immunosorbent assay (ELISA): the method of using a color reaction mediated by an enzyme bound to the Fc region of the antibody molecule. (P, colored product; S, substrate.)

the initial antibody solution can be diluted and still block viral infectivity (and thus formation of plaques). Neutralization is illustrated schematically in Fig. 7.10.

Inhibition of hemagglutination

Some methods for the measurement of antibody against viruses are based on the ability of the antibody to block some property of the virus. For example, it has been known since the first part of this century that many enveloped viruses will stick to red blood cells and cause them to agglutinate. This property of **hemagglutination** can be used as a crude measure of viral particle concentration in solution, as described in Chapter 9.

Many antibodies against enveloped viruses will inhibit virus-mediated agglutination of red blood cells, and this *hemagglutination inhibition (HI)* test can be used to measure antibody levels. The basic method was worked out long before a detailed understanding of the immune response was available, but it is based on the fact that many antibody molecules bind to the surface of viruses and physically mask them. If a virus that can cause hemagglutination is preincubated with an antibody to it, the virus will be coated with antibody and will not be able to stick to the red blood

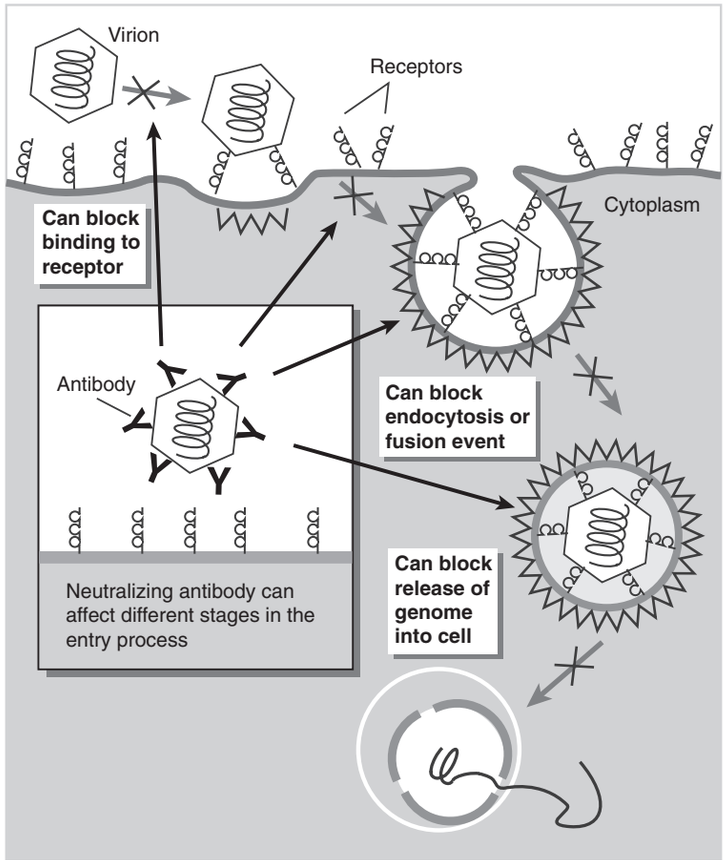

Fig. 7.10 Antibody neutralization of virus infectivity. Specific types of antibody molecules, called *neutralizing antibodies,* can bind to surface proteins of the virus and block one or another aspect of the early events of virus-cell recognition or effective internalization of the virus.

cells. This happens because the surface of the virus particle is relatively small, and once a protein molecule is stuck to it, that protein will block access to portions of the surface. If enough antibody sticks, the whole surface is obscured.

An experiment utilizing inhibition of hemagglutination (also called a HI test) is shown in Fig. 7.11. All that is required to measure a patient's immune response is a standard virus stock and blood serum. The basic procedure is as follows: Standard samples of red blood cells (e.g., guinea pig or chicken red blood cells for influenza virus) are mixed with a known amount of virus stock and different dilutions of an unknown antibody, which could be in a patient's serum. After a suitable period of time, the solution is gently shaken and subjected to low-speed centrifugation. If the red blood cells are agglutinated, the cells make a jelly-like clump and cannot sediment. Agglutination is characterized by a diffuse red or salmon-pink solution. If the red blood cells do not agglutinate, the cells pellet forms a red "button" at the bottom of the tube. The beauty of using HI is not accuracy; it is relative speed, ease, and low cost of performance, which is very important in small clinical laboratories, especially in developing countries.

Complement fixation

Serum complement is made up of a number of soluble proteins that are able to stick to cells bearing antibody–antigen complexes. As this binding occurs, the complement proteins undergo structural changes and finally, the last protein bound is activated to become a protease, which then lyses the cell. The ability of complement to bind to antibody–antigen complexes at the Fc region of the

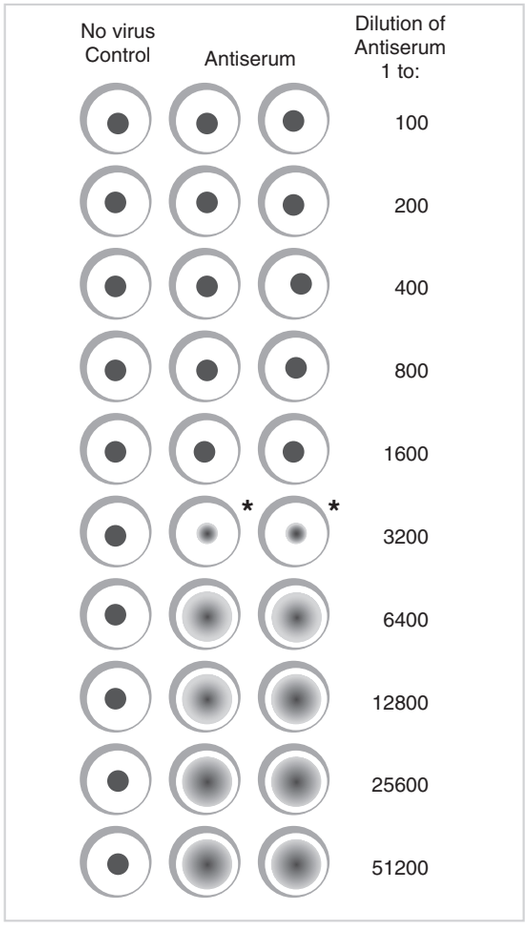

Fig. 7.11 The hemagglutination inhibition assay for measuring antibody against a virus in serum. The assay is carried out by mixing constant amounts of a known hemagglutinating virus with serial dilutions of serum; then the virus-serum mixture is added to red blood cells. Low dilutions of serum result in sequestering the virus so that it is not available for hemagglutination, and red blood cells in the wells pellet to the bottom under low centrifugal fields. Higher dilutions of the antiserum dilutes the antibody concentration to a point where enough virus remains to cause a positive hemagglutinin reaction. If there were more antibody in the serum, a higher dilution would be required to accomplish this. Thus, the hemagglutination inhibition titer of the serum is a measure of how far it can be diluted and still block the hemagglutinin reaction. This is a measure of antibody concentration. In the example shown, a 1 : 3200 dilution of the original sample (asterisks) was the last one in which agglutination was inhibited. This is the endpoint of the antiserum dilution. Since a 1 : 3200 dilution was the endpoint, there were 3200 hemagglutination inhibition units in the original stock. (Based on a figure in Dimmock, N.J., and Primrose, S.B. *Introduction to Modern Virology*, 4th edn. Boston: Blackwell Science, 1994.)

antibody is termed fixation because once bound, the complement is no longer free in solution. This property can be used as a relatively simple and inexpensive method to measure antibody–antigen reactions called *complement fixation* (CF) titration.

In a CF assay, sheep red blood cells are used to make an antibody against their surface proteins, often in a horse, goat, or other large animal. The red blood cells are then "standardized" so that when a specific amount of antibody is added to them and the mix is incubated with guinea pig complement, the red blood cells lyse. Lysis of the red blood cells is readily assayed because when a solution of lysed red blood cells is centrifuged at low speed, the solution will stay red because there are no cells to take the hemoglobin to the bottom of the tube to form a pellet.

After the red blood cells, anti-red blood cell serum, and complement are standardized, they can be stored for relatively long periods in the cold. When they are used to assay an antibody–antigen reaction, the following process is carried out. Serial dilutions of either a solution of antibody of unknown strength and a fixed amount of known virus, *or* a solution with an unknown amount of virus and a fixed amount of known antibody, are incubated together. Then they are mixed with a known amount of guinea pig complement. If an antibody–antigen complex has formed, the complement will be *fixed* by it. If not, the complement will stay in solution. If there is an intermediate level of complex, then some complement will be fixed and some will be free.

Following incubation of the unknown antibody–antigen mix with the known amount of complement, the whole "mess" is incubated with standard amounts of red blood cells and anti-red

blood cell antibody. If all the complement is fixed, there will be no lysis of the red blood cells. If some is fixed, there will be partial lysis of the red blood cells. If none is fixed, there will be complete lysis. Measurement of the degree of lysis (by measuring the amount of red color in solution following low-speed centrifugation) can be used to measure the amount of unknown antibody–antigen reaction and provides the CF titer.

Like HI, this method is not extremely precise or sensitive, but it is cheap, fast, and requires few expensive pieces of equipment. It is an ideal method for getting quick results in small laboratories and in Third World clinical laboratories. It is still used in all modern hospitals.

QUESTIONS FOR CHAPTER 7

1 Which of the following statements is/are true?
- **a** The only region in the body where a virus-infected cell can interact with T cells is in the lymph nodes.
- **b** Virion surface proteins tend to elicit a stronger immune response during the course of natural infection than do internal components of the virion.
- **c** Epitope-containing antigens must be digested to single amino acids and reassembled at the surface in the presence of histocompatibility antigens in order to provoke immunity.

2 Why are soluble antibodies (the products of the humoral response) good antiviral agents?

3 What are the roles of the following cells in the vertebrate immune response?
- **a** B cells
- **b** Helper T cells ($CD4^+$)
- **c** Cytotoxic T cells ($CD8^+$)

4 What protein structural features are involved in the antigenic nature of epitopes?

5 What steps occur in the immune response following the primary infection of a vertebrate by a virus?

6 Assume you know that for a particular virus, gene A codes for a transcriptional activator, gene B for an origin-binding protein, and gene C for a capsid protein. Following a normal infection in an animal, what would most likely generate a neutralizing antibody?

7 What are some of the problems that arise in considering vaccination strategies for viral diseases?