CHAPTER 2

Immunization and Adaptive Immunity: The Immune Response Arc and Immune Effectors

Highlights

- Adaptive (or acquired) immunity involves a "learned" response to specific antigens rather than a response to conserved molecular patterns.
- The adaptive immune system continuously samples antigenic epitopes, determines whether they are self or nonself, and then mounts an immune response to eliminate foreign antigens.
- Conversion of an antigenic stimulus to an immune response involves activation of immune cells, particularly T and B lymphocytes, and development of immunologic memory for that specific antigen.
- The adaptive immune response comprises antibody-mediated responses, cell-mediated defense primarily coordinated by T lymphocytes, and combined antibody and cellular mechanisms.
- Cell-mediated responses (particularly T helper [Th]-1, Th2, and Th17 responses) are relevant to the immunopathology of several intraocular inflammatory conditions.

Definitions

The term *antigen* refers to a substance recognized by the immune system. The term *epitope* refers to each specific portion of an antigen to which an antibody molecule can bind. Antigenic epitopes exist on both native self tissues and foreign, or nonself, tissues. A complex, 3-dimensional protein has multiple antigenic epitopes that the immune system can recognize, as well as many other sites that remain unrecognizable and thus invisible to the immune system.

The adaptive immune response, unlike the innate immune response (discussed in Chapter 1), is a learned response to highly variable but specific antigenic epitopes rather

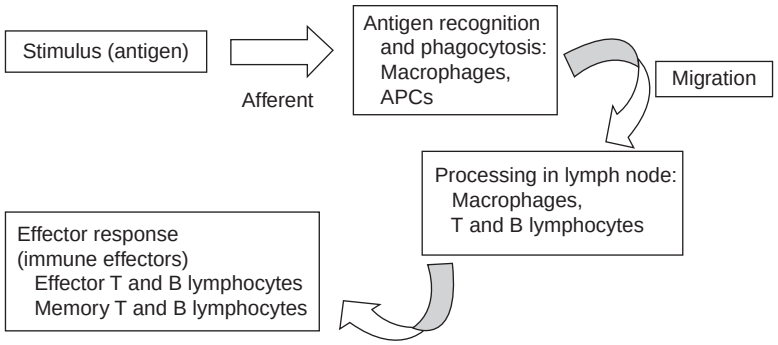

Figure 2-1 The immune response arc. APCs = antigen-presenting cells.

than a response to conserved molecular patterns. The purpose of the adaptive immune system is to continuously sample antigenic epitopes, determine whether they are self or non-self, and then mount an immune response to eliminate foreign antigens. The immune response arc—the interaction between antigen and the adaptive immune system—can be divided into 3 phases: afferent, processing, and effector (Fig 2-1).

Phases of the Immune Response Arc

Afferent Phase

The afferent phase of the immune response arc comprises the initial recognition, transport, and presentation of antigenic substances to the adaptive immune system. Recognition starts with antigen-presenting cells (APCs), specialized cells that bind antigen at a peripheral site. After the APC receives stimulatory signals (ie, complement), phagocytosis of the antigen occurs, as discussed in Chapter 1. Following ingestion of antigen, APCs migrate via afferent lymphatics to regional lymph nodes.

In the APCs, enzymatic digestion of proteins within endocytic vesicles produces antigenic epitope fragments of 7–11 amino acids (Fig 2-2). Each antigenic fragment binds to a groove-shaped human leukocyte antigen (HLA) peptide residing on the APC surface. The combination of antigenic peptide and HLA protein is recognized by the T-lymphocyte receptors CD4 and CD8. HLA molecules differ in their capacity to bind various antigenic peptide fragments within their grooves; thus, the HLA type determines the array of peptide antigens that can be presented to T lymphocytes. Specific HLA alleles are important risk factors for certain forms of uveitis. See Chapter 4 for further discussion of HLA molecules and disease susceptibility.

HLA peptides are part of a family of cell surface glycoproteins called *major histocompatibility complex (MHC) proteins.* In humans, MHC proteins are termed *human leukocyte antigen (HLA) molecules*. HLA class I molecules (ie, HLA-A, -B, and -C) serve as the antigen-presenting platform for $CD8^+$ T lymphocytes, which are essentially cytotoxic T cells (see Fig 2-2A). Class I molecules are present on almost all nucleated cells and generally function to process peptide antigens synthesized by the host cell. If these presented antigens are

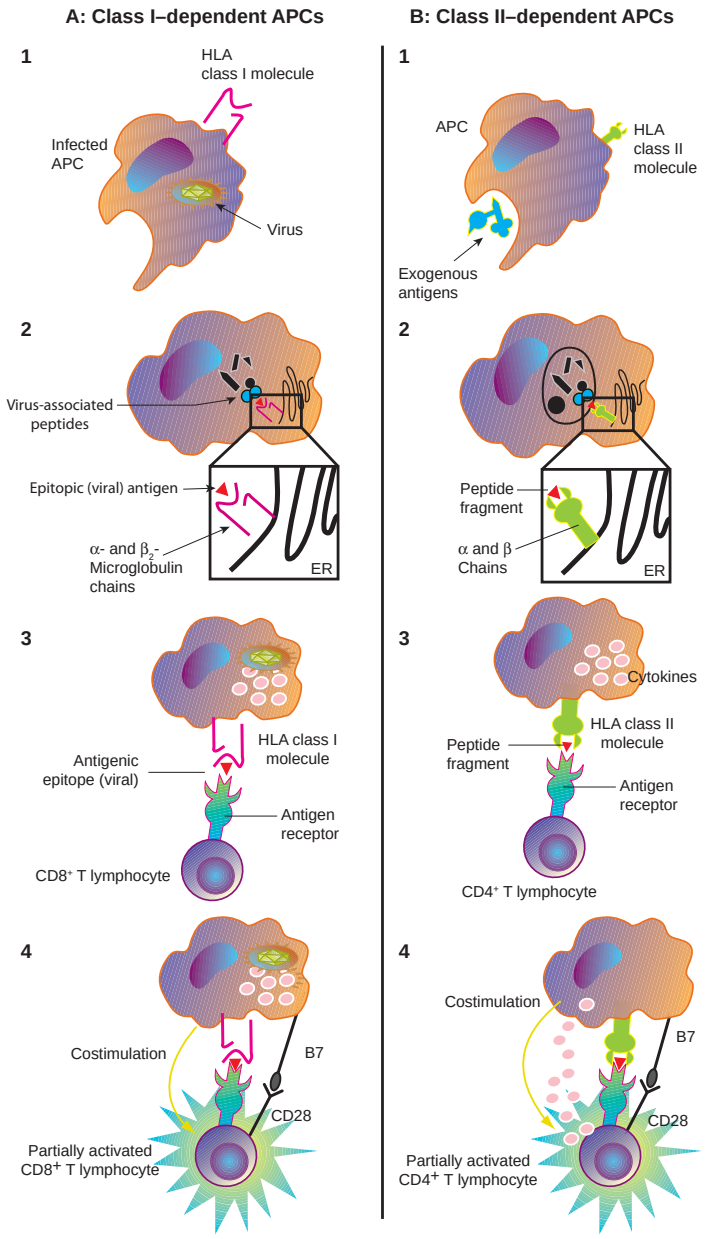

Figure 2-2 **A,** Class I–dependent APCs. **1,** APC is infected by a virus, which causes the cell to synthesize virus-associated peptides that are present in the cytosol. **2,** The viral antigen is transported through specialized transport systems into the endoplasmic reticulum (ER), where the viral antigen encounters human leukocyte antigen (HLA) class I molecules. The antigenic fragment binds to the groove formed by the α chain of the HLA class I molecule. Unlike class II molecules, the second chain, called *β_2-microglobulin,* is constant among all class I molecules. **3,** The CD8 T-lymphocyte receptor recognizes the fragment–class I complex displayed on the cell membrane. **4,** With the help of costimulatory molecules such as CD28-B7 and cytokines, the $CD8^+$ T lymphocyte becomes primed, or partially activated. A similar mechanism is used to recognize tumor antigens that are produced by cells after malignant transformation. **B,** Class II–dependent APCs. **1,** APCs endocytose exogenous antigens into the ER. **2,** In the ER, the antigen is digested, generating peptide fragments that bind to the groove formed by the α and β chains of the HLA class II molecule. **3,** The $CD4^+$ T-lymphocyte receptor recognizes the fragment–class II complex. **4,** With the help of costimulatory molecules such as CD28-B7 and cytokines, the $CD4^+$ T lymphocyte becomes primed, or partially activated. *(Illustration by Barb Cousins, modified by Joyce Zavarro.)*

recognized as self (ie, normal host protein), no immune reaction occurs. However, if there is an alteration of the normal host peptide (termed *altered self*), by tumor or viral peptides after host cell invasion, an immune response is initiated. A viral infection, a neoplasm, or simply a genetic mutation that alters protein structure may induce autoimmunity by stimulating an inappropriate immune response to normal host proteins.

HLA class II molecules (ie, HLA-DR, -DP, and -DQ) serve as the antigen-presenting platform for $CD4^+$, or *helper,* T lymphocytes (see Fig 2-2B). The antigen receptor on the helper T lymphocyte recognizes peptide antigens only if the antigens are presented with class II molecules simultaneously. Only certain cell types express HLA class II molecules. Macrophages and dendritic cells are the most important of these types, although B lymphocytes also may function as class II–dependent APCs, especially within a lymph node. Class II–dependent APCs are considered the most efficient APCs for processing extracellular protein antigens; that is, antigens that have been phagocytosed from the external environment (eg, bacterial or fungal antigens).

Processing Phase

The conversion of an antigenic stimulus into an immunologic response occurs through *priming* of naive B and T lymphocytes (lymphocytes that have not yet encountered their specific antigen) within lymph nodes and the spleen. Processing involves regulation of the interaction between antigen and naive lymphocytes, followed by lymphocyte *activation,* which consists of lymphocyte proliferation and differentiation (Fig 2-3).

Preconditions necessary for processing

The principal cell type for immune processing is the $CD4^+$ T lymphocyte. These lymphocytes have a receptor that detects antigen only upon formation of a trimolecular complex consisting of an HLA class II molecule, a processed antigenic fragment, and a T-lymphocyte antigen receptor (see Fig 2-2B). The $CD4^+$ molecule stabilizes binding and enhances signaling between the HLA complex on the APC and the T-lymphocyte receptor. When helper T lymphocytes recognize their specific antigen, they become primed, or partially activated, acquiring new functional properties, including cell division, cytokine synthesis, and cell membrane expression of *accessory molecules,* such as cell adhesion molecules and costimulatory molecules. The synthesis and release of immune cytokines, especially interleukin (IL)-2, by T lymphocytes is crucial for the progression of initial activation and the functional differentiation of T lymphocytes through autocrine stimulation.

Helper T-lymphocyte differentiation

Prior to priming, $CD4^+$ T lymphocytes are classified as T helper (Th)-0 cells. These cells can differentiate into 1 of at least 4 functional subtypes—Th1, Th2, Th17, or T regulatory (Treg)—based on the pattern of cytokines to which they are exposed (Fig 2-4). Each helper T-cell subtype, in turn, produces a characteristic profile of cytokines that is regulated by the expression of subtype-specific transcription factors.

Th1 cells participate in the elimination of intracellular pathogens as well as in cell-mediated and delayed hypersensitivity reactions. Th1 cells secrete interferon gamma (IFN-γ), IL-2, and tumor necrosis factor α and β (TNF-α, TNF-β).

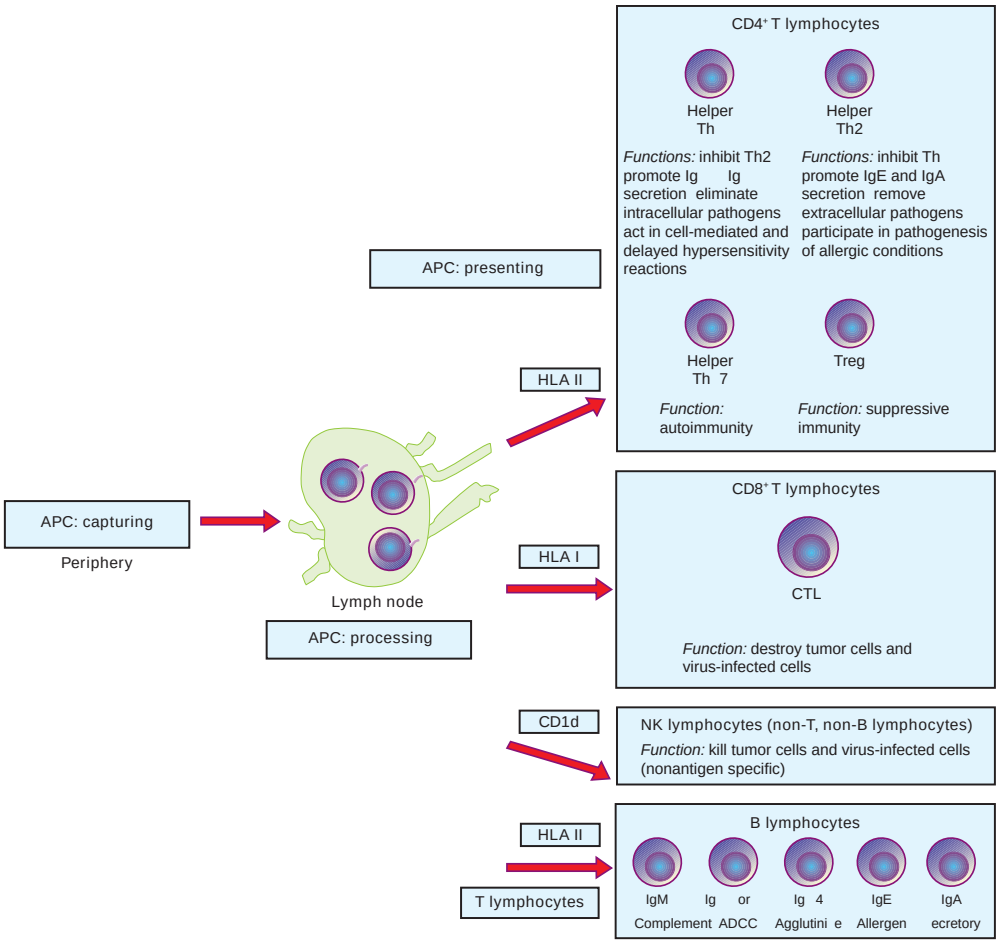

Figure 2-3 Schematic illustration of immune processing of antigen within the lymph node. On exposure to antigen and APCs within the lymph node, the 3 major lymphocyte subsets—B lymphocytes, $CD4^+$ T lymphocytes, and $CD8^+$ T lymphocytes—and natural killer (NK) (non-T, non-B) lymphocytes are activated to release specific cytokines and perform particular activities. Antigen presented by HLA class II molecules stimulates $CD4^+$ T lymphocytes to differentiate into 1 of at least 4 subtypes: T helper (Th)-1, Th2, Th17, or T regulatory (Treg).

Naive $CD8^+$ T cells become cytotoxic T lymphocytes (CTLs), and non-T, non-B lymphocytes become NK lymphocytes. B lymphocytes are stimulated to produce 1 of the various antibody isotypes, whose functions may include complement activation, antibody-dependent cellular cytotoxicity (ADCC), agglutination, allergen recognition, and/or secretory release. Ig = immunoglobulin. *(Illustration by Barb Cousins, modified by Joyce Zavarro.)*

Th2 cells are involved in the clearance of extracellular pathogens and play an important role in the pathogenesis of allergic conditions such as asthma. Th2 cells produce IL-4, IL-5, and IL-13 but not Th1 cytokines.

Th17 cells contribute to immunity against certain extracellular bacteria and fungi and play a role in the defense of mucosal surfaces. Th17 cells produce IL-17, IL-21,

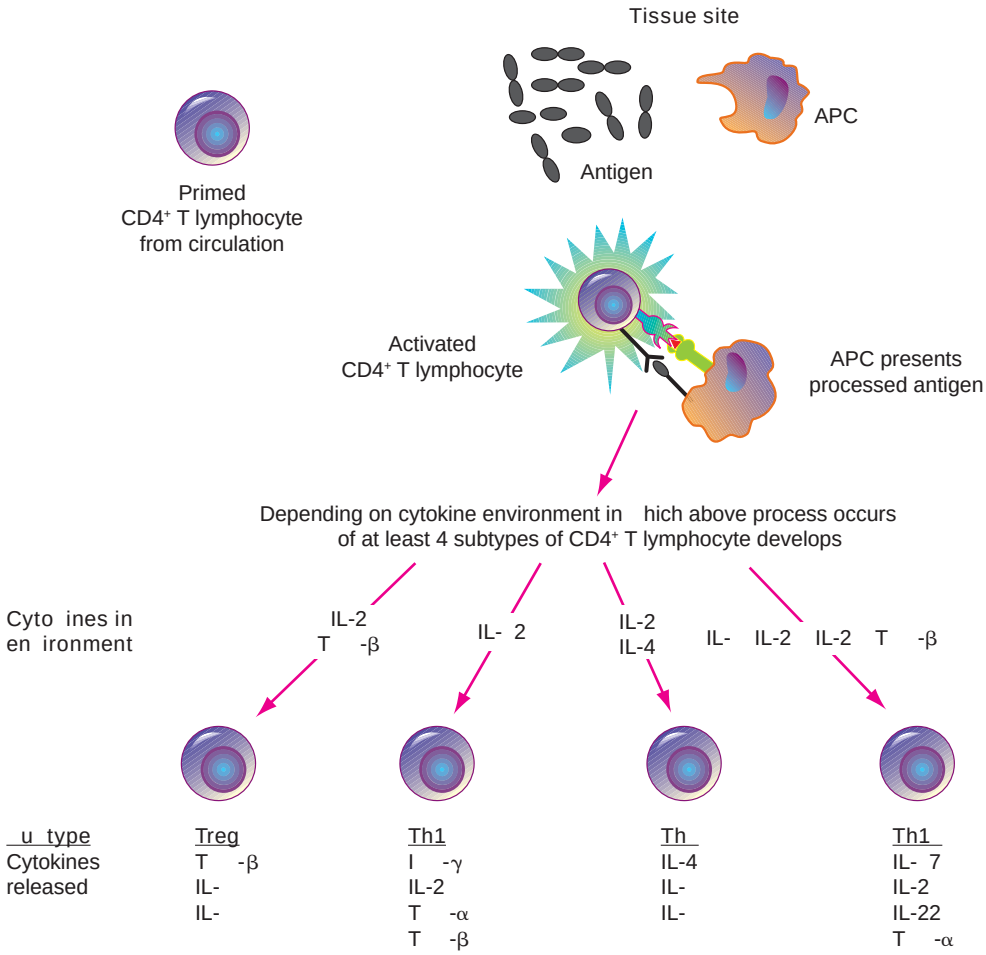

Figure 2-4 Schematic representation of CD4⁺ T-lymphocyte development. After initial priming in the lymph node, CD4⁺ T lymphocytes enter the tissue site, where they again encounter APCs containing processed antigen. Upon restimulation and depending on the cytokines present in the local environment at the time of restimulation, CD4⁺ T lymphocytes become activated into 1 of at least 4 subtypes. Tregs suppress other T-cell responses. Th1 lymphocytes are the classic delayed hypersensitivity effector cells and mediate interferon gamma (IFN-γ)–driven responses. Th2 lymphocytes are thought to be less intensively inflammatory and have been associated with granuloma formation in response to parasite-derived antigens, as well as manifestations of atopic diseases. Th17 lymphocytes mediate and sustain inflammation. IL = interleukin; TGF = transforming growth factor. *(Illustration developed by Russell W. Read, MD, PhD.)*

IL-22, and TNF-α, in association with several transcription factors, including retinoic acid receptor–related orphan receptor-γt and receptor-α (ROR-γt and -α). Dysregulation of Th17 proinflammatory cytokines IL-17 and IL-22 has been implicated in the pathogenesis of systemic inflammatory diseases, such as psoriasis, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, Sjögren syndrome, Behçet disease, and systemic lupus erythematosus, as well as in uveitis and scleritis.

Treg cells are essential for maintaining peripheral tolerance to autoantigens (also called *self antigens*), thereby preventing autoimmune diseases and limiting chronic inflammatory diseases. Treg cells suppress excessive immune responses deleterious to the host and downregulate autoreactive T cells. Treg cells are identified by their simultaneous expression of CD4, CD25, and Foxp3. The suppressive cytokines transforming growth factor β and IL-10 have been implicated as active players in the effector function of Treg cells as regulators of inflammation.

An imbalance between regulatory mechanisms that inhibit the immune system and proinflammatory responses is thought to be the underlying cause of uveitis and many other immune-mediated diseases. The cytokine profiles produced by these various helper T-cell subtypes determine subsequent immune processing, B-lymphocyte antibody synthesis, and cell-mediated effector responses. For example, IFN-γ produced by Th1 lymphocytes inhibits the Th2 response, whereas IL-4 produced by Th2 lymphocytes inhibits the Th1 response. The process determining whether a Th1 or a Th2 response develops with exposure to a specific antigen is not entirely understood, but presumed variables include cytokines preexisting in the microenvironment, the nature and amount of antigen encountered, and the type of APC involved. For example, IL-12, which is produced by macrophage APCs, might preferentially induce Th1 responses.

Abbas AK, Lichtman AH, Pillai S. *Cellular and Molecular Immunology*. 9th ed. Elsevier/Saunders; 2018.

Amadi-Obi A, Yu CR, Liu X, et al. Th17 cells contribute to uveitis and scleritis and are expanded by IL-2 and inhibited by IL-27/STAT1. *Nat Med.* 2007;13(6):711–718.

Caspi RR. Understanding autoimmunity in the eye: from animal models to novel therapies. *Discov Med.* 2014;17(93):155–162.

B-lymphocyte activation

A major function of helper T lymphocytes is B-lymphocyte activation. B lymphocytes are responsible for producing antibodies, or immunoglobulin (Ig) molecules (glycoproteins that bind to the specific antigens or epitopes that induced their synthesis). These antibodies contain an epitope-specific binding site, termed *paratope,* on the Fab (*f*ragment, *a*ntigen-*b*inding) portion of the molecule. B lymphocytes begin as naive lymphocytes; IgM and IgD are expressed on their cell surface and serve as B-lymphocyte antigen receptors. Through these surface antibodies, B lymphocytes detect epitopes on intact antigens *without* the requirement of antigen processing by APCs. After appropriate stimulation of the B-lymphocyte antigen receptor, helper T lymphocyte–B lymphocyte interaction occurs, leading to further B-lymphocyte activation and differentiation. B lymphocytes acquire new functional properties, such as cell division, cell surface expression of accessory molecules, and synthesis of large quantities of antibody. The terminal form of B-lymphocyte differentiation is the plasma cell, which secretes antibodies, or immunoglobulins. Activated B lymphocytes acquire the ability to switch their surface IgM antibodies to another immunoglobulin class (eg, IgG, IgA, or IgE). This class shift requires a molecular change in the immunoglobulin heavy chain and is regulated by specific cytokines released by the helper T lymphocyte. For example, the cytokine IFN-γ induces a switch from IgM to IgG1 production in an antigen-primed B lymphocyte, whereas treatment with IL-4 induces a switch from IgM to IgE.

Effector Phase

The elimination or neutralization of foreign antigen, which is the purpose of the adaptive immune response, is accomplished during the effector phase. Antigen-specific effectors exist in 2 major subsets (Fig 2-5):

- T lymphocytes, including delayed hypersensitivity and cytotoxic T lymphocytes
- B lymphocytes and the antibodies produced by their derived plasma cells

A third subset of effector lymphocytes—grouped as non-T, non-B lymphocytes—includes natural killer cells, lymphokine-activated cells, and killer cells (see Fig 2-3).

Effector lymphocytes require 2 exposures to antigen for maximum effectiveness. The initial (*priming* or *activation*) exposure occurs in the lymph node. The second *(restimulation)* exposure occurs in the peripheral tissue from which the antigen originated.

Delayed hypersensitivity T lymphocytes usually express the cell marker CD4 and release IFN-γ and TNF-β. Cytotoxic T lymphocytes express CD8 and kill tumor cells and virus-infected host cells. See the section Lymphocyte-Mediated Effector Responses for further discussion of these cells.

The B-lymphocyte effector response is mediated by antibodies produced by plasma cells. Antibodies are released into the efferent lymph fluid (downstream from lymph nodes), draining into the venous circulation. Once bound to their specific epitope, antibodies

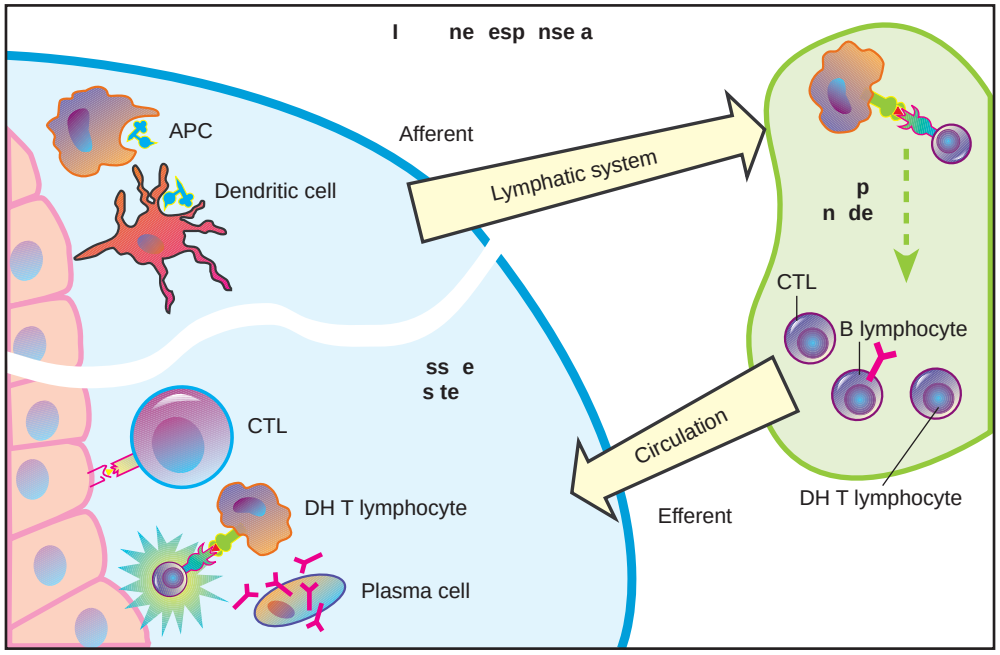

Figure 2-5 Schematic representation of effector mechanisms during the adaptive immune response. The tissue site *(left)* is not only where the immune response is initiated but is also ultimately where the immune response arc is completed—when effectors encounter antigen within tissue after their release from the lymph node *(right)* into the circulation. The 3 most important effector mechanisms of adaptive immunity are CTLs, delayed hypersensitivity (DH) T lymphocytes ($CD4^+$ T lymphocytes), and antibody-producing plasma cells derived from B lymphocytes. *(Illustration by Barb Cousins, modified by Joyce Zavarro.)*

mediate a variety of effector activities, including opsonization for phagocytosis and complement activation via the classical pathway.

The Immune Response Arc and Primary or Secondary Immune Response

Immunologic memory is the most distinctive feature of adaptive immunity. Protective immunization is the prototypical example of this powerful phenomenon.

Differences Between Primary and Secondary Responses

The immune response that occurs on the second or subsequent encounter with an antigen *(anamnestic response)* is regulated differently from that occurring on the first encounter. During the processing phase of the primary response, relatively rare antigen-specific B lymphocytes (perhaps 1 in 100,000 B lymphocytes) and T lymphocytes (perhaps 1 in 10,000 T lymphocytes) must come in contact with appropriately presented antigen. Stimulation of these cells from a completely resting and naive state then occurs, a process that requires days. Following the primary response, various events occur that set the stage for a subsequent rapid and robust secondary response:

- Following stimulation, lymphocytes divide, dramatically increasing the population of antigen-responsive T and B lymphocytes *(clonal expansion),* and migrate to other sites of potential encounter with antigen.
- Upon removal of antigen, T and B lymphocytes activated during the primary response gradually return to a resting state but are no longer naive. They retain the capacity to become reactivated within 12–24 hours of antigen exposure and thus are termed *memory cells.*
- Memory lymphocytes express higher levels of certain cell adhesion molecules, such as integrins, than do naive lymphocytes. Expression of these cell adhesion molecules facilitates *homing,* or migration into target tissues.
- IgM produced during the primary response may be too large to leak passively into a peripheral site. Following antibody-class switching, IgG or other isotypes can leak passively into or be produced at the site; thus, they can immediately bind to antigen and trigger a rapid secondary response.
- In some cases, such as in mycobacterial infection, low doses of antigen may remain in the node or site, producing a chronic, low-level antigenic stimulation of T and B lymphocytes. See Clinical Example 2-1.

CLINICAL EXAMPLE 2-1

Primary and Secondary Response to Tuberculosis

The afferent phase of the primary response begins when alveolar macrophages ingest *Mycobacterium tuberculosis* within the lung and then transport the organisms to the hilar lymph nodes. As T and B lymphocytes

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are primed, the hilar nodes become enlarged. The effector phase begins when the primed T lymphocytes recirculate and enter the infected lung. The T lymphocytes interact with macrophage-ingested bacteria, and cytokines are released that activate neighboring macrophages to differentiate into epithelioid cells, which fuse to form giant cells, eventually forming caseating granulomas. Meanwhile, some effector T lymphocytes become inactive memory T lymphocytes.

A secondary response in the skin is the basis of the tuberculin skin test used to diagnose tuberculosis (TB). The afferent phase of the secondary response begins when a purified protein derivative (PPD) reagent (antigens purified from nontuberculous mycobacteria) is taken up by dermal macrophages after injection. The secondary processing phase begins when the PPD-stimulated macrophages migrate into the draining lymph node, where they encounter memory T lymphocytes, which then reactivate.

The secondary effector phase commences when the reactivated memory T lymphocytes recirculate and home to the dermis, where they encounter additional antigen and macrophages at the injection site, causing the T lymphocytes to become fully activated and release cytokines. Within 24–72 hours, the cytokines induce infiltration of additional lymphocytes and monocytes as well as fibrin clotting. This process produces the typical indurated dermal lesion of the TB skin test, called the *tuberculin form* of delayed hypersensitivity.

Effector Responses and Mechanisms of Adaptive Immunity

In 1962, Gell and Coombs elaborated on 4 mechanisms of adaptive immune-triggered inflammatory responses, creating a classification of allergic reactions comprising types I through IV:

- anaphylaxis
- antibody-dependent cellular cytotoxicity
- immune complex-mediated reactions
- cell-mediated reactions

Familiarity with Gell and Coombs' classifications is important for understanding older literature. However, it is more accurate to divide the effector responses of adaptive immunity into 3 main categories:

- antibody-mediated effector responses
- lymphocyte-mediated effector responses (delayed hypersensitivity, cytotoxic lymphocytes)
- combined antibody and cellular mechanisms

Delves PJ, Martin SJ, Burton DR, Roitt IM. *Roitt's Essential Immunology.* 13th ed. Wiley-Blackwell; 2017.

Owen JA, Punt J, Stranford SA. *Kuby Immunology.* 7th ed. WH Freeman; 2013.

Antibody-Mediated Effector Responses

Structural and functional properties of antibody molecules

Structural features of immunoglobulins There are 5 major classes or isotypes of immunoglobulins (M, G, A, E, and D). IgG and IgA can be further divided into subclasses (IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2). The basic immunoglobulin structure, referred to as a *monomer,* is composed of 4 covalently bonded glycoprotein chains (Fig 2-6):

- 2 identical light chains, either kappa (κ) or lambda (λ)
- 2 identical heavy chains

Each monomer is approximately 150,000–180,000 Da. The type of heavy chain defines the specific immunoglobulin isotype or subclass. IgM can form pentamers or hexamers in vivo, and IgA can form dimers in secretions, so the in vivo molecular size of these 2 classes is much larger than that of the others.

Antibodies contain regions called *domains* that carry out the specific functions of the antibody molecule. The Fab region (2 of which are present on each molecule) contains the antigen-binding domain, called the *variable region.* The opposite end of the molecule, on the heavy chain portion, contains the attachment site for effector cells (the *Fc* [*f*ragment *c*rystallizable] *portion*). It also contains the site of other effector functions, such as complement fixation (for IgG3) or binding to a secretory component for transportation through epithelia and secretion into tears (for IgA). Table 2-1 summarizes key differences between immunoglobulin isotypes.

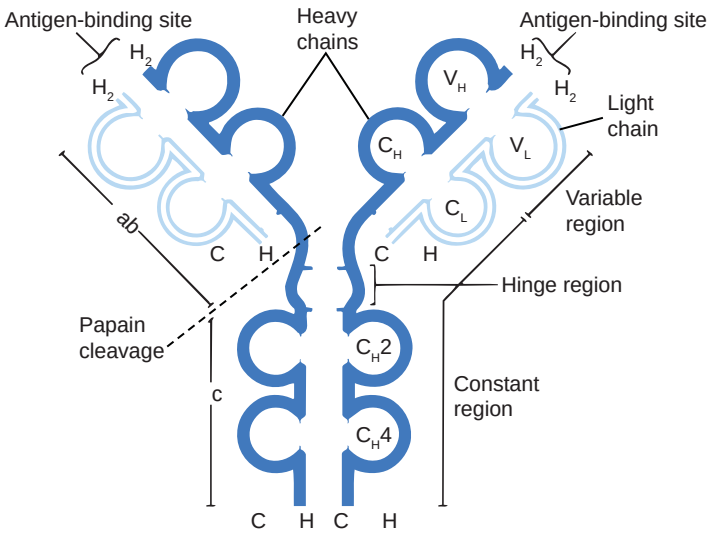

Figure 2-6 Schematic representation of an immunoglobulin molecule. The *solid lines* indicate the 2 identical heavy chains; the *light-blue double lines* indicate the identical light chains; *-ss-* indicates intrachain and interchain covalent disulfide bonds. Fab = *f*ragment, *a*ntigen-*b*inding region; Fc = *f*ragment *c*rystallizable region (attachment site for effector cells). *(Reproduced with permission from* Dorland's Illustrated Medical Dictionary. *32nd ed. Elsevier/Saunders; 2012:921.)*

Table 2-1 Structural and Functional Properties of Immunoglobulin Isotypes

Immunoglobulin (Ig) Isotype	Percentage of Total Serum Immunoglobulins	Structural Features	Functions
IgD	<1%	Mostly on surface of B lymphocytes	B-lymphocyte antigen receptor
IgM	5%	Mostly on surface of B lymphocytes or intravascular	B-lymphocyte antigen receptor, agglutination, neutralization, intravascular cytolysis, classical complement pathway activation
IgG	77%	Intravascular, in tissues; crosses placenta	Cytolysis (IgG1, IgG3), ADCC (IgG2, IgG3), agglutination (IgG3), neutralization (IgG4), classical complement pathway activation (IgG1, IgG3)
IgE	<1%	Mostly in skin or mucosa; bound to mast cells	Mast cell degranulation
IgA	18%	In mucosal secretions, binds secretory component in subepithelial tissues for transepithelial transport and protection from proteolysis	Mucosal immunity, neutralization, alternative complement pathway activation

ADCC = antibody-dependent cellular cytotoxicity.

Functional properties of immunoglobulins Immunoglobulin isotypes differ in how they mediate antibody effector functions. Human IgM and IgG3 are good complement activators, whereas IgG4 is not. Only IgA can bind the secretory component and be actively passed into mucosal secretions. The importance of these differences is that 2 antibodies with identical capacity to bind to an antigen—but of different isotype—will produce different effector and inflammatory outcomes.

Terminology

Clonality Each B cell creates a unique Fab fragment that recognizes a single antigenic configuration. Clonal expansion, or proliferation of an individual B lymphocyte via cell division, results in a population of identical B cells that recognize the same epitope, termed a *monoclonal* population. Biologic drugs such as infliximab, adalimumab, and rituximab are examples of recombinant monoclonal antibodies that specifically target immune system molecules (see Chapter 6). However, the typical immune response is a *polyclonal response* since many individual B cells will recognize various epitopes on the same substance, and each B cell will undergo simultaneous clonal expansion.

Idiotypes Because they are proteins, antibodies themselves can be antigenic. Their antigenic sites are called *idiotopes,* as distinguished from *epitopes,* the antigenic sites on foreign molecules. Anti-idiotypic antibodies may function as feedback mechanisms for immune regulation and have clinical significance for modern biologic therapies. Infliximab, for example, is a monoclonal chimeric (mouse and human) antibody to TNF-α that is used to treat some forms of uveitis. Efficacy of this drug may be limited by the development of anti-idiotypic antibodies that neutralize the antigen-binding site for TNF-α.

Infiltration of B lymphocytes into tissues and local production of antibody

B-lymphocyte infiltration B lymphocytes can infiltrate the site of an immunologic reaction in response to persistent antigenic stimulus. If the process becomes chronic, plasma cell formation occurs, with local production of antibody specific for the inciting antigen(s). Assessment of local antibody production can serve as a diagnostic test (see Clinical Example 2-2) when the antigen is known or suspected, as in the case of a presumed infection.

CLINICAL EXAMPLE 2-2

The Clinical Significance of Local Antibody Production

Distinguishing between local production of antibody and passive leakage of antibody from the blood to the intraocular compartment involves determination of the *Goldmann-Witmer (GW) coefficient.* This is calculated by comparing the ratio of specific IgG antibody present in intraocular fluid to the total IgG level in intraocular fluid versus the ratio of specific IgG level in serum to the total IgG level in serum:

$$\text{GW Coefficient} = (\text{Intraocular Specific IgG}/\text{Intraocular Total IgG}) / (\text{Serum Specific IgG}/\text{Serum Total IgG})$$

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Quentin and Reiber used the GW coefficient to demonstrate that aqueous from eyes with Fuchs uveitis syndrome had markedly elevated intraocular IgG titers to rubella virus compared with levels found in controls. The average GW coefficient was 20.6 in patients with Fuchs uveitis syndrome, compared with less than 1.5 in controls.

Quentin CD, Reiber H. Fuchs heterochromic cyclitis: rubella virus antibodies and genome in aqueous humor. *Am J Ophthalmol.* 2004;138(1): 46–54.

Local antibody production within a tissue and chronic inflammation Persistence of antigen within a site, coupled with infiltration of specific B lymphocytes and local antibody formation, can produce a chronic inflammatory reaction called the *chronic Arthus reaction*. The histologic pattern often demonstrates lymphocytic infiltration, plasma cell infiltration, and granulomatous features. This type of chronic inflammation may contribute to the pathophysiology of certain chronic autoimmune disorders, such as rheumatoid arthritis, which feature formation of pathogenic antibodies.

Abbas AK, Lichtman AH, Pillai S. *Cellular and Molecular Immunology.* 9th ed. Elsevier/Saunders; 2018.

Mousa HM, Saban DR, Foster CS, Cordero-Coma M, Streilein JW. Allergy and immune-mediated tissue injury. In: Albert DM, Miller JW, Azar DT, Young LH, eds. *Albert and Jakobiec's Principles and Practice of Ophthalmology.* 4th ed. Springer; 2022:837–855.

Lymphocyte-Mediated Effector Responses

Delayed hypersensitivity T lymphocytes

Delayed hypersensitivity (DH) represents the prototypical adaptive immune mechanism for lymphocyte-triggered inflammation. It is especially powerful in secondary immune responses. Previously primed DH $CD4^+$ T lymphocytes leave the lymph node, home to local tissues where antigen persists, and become activated by restimulation with the specific priming antigen and HLA class II–expressing APCs. Fully activated DH T lymphocytes secrete mediators and cytokines (see Fig 2-4), leading to the recruitment and activation of macrophages and/or other nonspecific leukocytes. The term *delayed* for this type of hypersensitivity refers to the fact that the reaction becomes maximal 12–48 hours after antigen exposure.

Just as helper T lymphocytes can be divided into 3 subsets—Th1, Th2, and Th17—according to the spectrum of cytokines they secrete, DH T lymphocytes can also be grouped by the same criteria. Experimentally, Th1 cytokines, especially IFN-γ (also known as *macrophage-activating factor*) and TNF-α, activate macrophages to secrete inflammatory mediators and kill pathogens, thus amplifying inflammation. Th1-mediated DH effector mechanisms, therefore, are thought to produce the following effects:

- the classic DH reaction (eg, the PPD skin reaction)
- immune responses to intracellular infections (eg, to mycobacteria or *Pneumocystis* organisms)

Table 2-2 Ocular Inflammatory Diseases Likely Involving Th1-Mediated Delayed Hypersensitivity Effector Mechanisms

Site	Disease	Presumed Antigen
Conjunctiva	Contact hypersensitivity to contact lens solutions	Thimerosal or other chemicals
	Giant papillary conjunctivitis	Unknown
	Phlyctenulosis	Bacterial antigens
Cornea and sclera	Chronic allograft rejection	Histocompatibility antigens
	Marginal infiltrates of blepharitis	Bacterial antigens
	Disciform keratitis after viral infection	Viral antigens
Anterior uvea	Acute anterior uveitis	Uveal autoantigens, bacterial antigens
	Sarcoidosis-associated uveitis	Unknown
	Idiopathic intermediate uveitis	Unknown
Retina and choroid	Sympathetic ophthalmia	Uveal or retinal autoantigens
	Vogt-Koyanagi-Harada syndrome	Uveal or retinal autoantigens
	Birdshot chorioretinopathy	Retinal or uveal autoantigens
Orbit	Acute thyroid orbitopathy	Probably thyrotropin receptor
	Giant cell arteritis	Unknown

Th = T helper.

- immune responses to fungal infections
- most forms of severe T-lymphocyte–mediated autoimmune diseases
- chronic transplant rejection

Table 2-2 summarizes the ocular inflammatory diseases thought to require a major contribution of Th1-mediated DH effector mechanisms.

The Th2 subset of DH cells secretes IL-4, IL-5, and other cytokines. IL-4 can induce B lymphocytes to synthesize IgE, and IL-5 can recruit and activate eosinophils within a site. IL-4 can also induce granuloma formation in response to parasite-derived antigens. Thus, Th2-mediated DH mechanisms are thought to play a major role in the following:

- response to parasitic infections
- late-phase responses in allergic reactions
- asthma
- atopic dermatitis or other manifestations of atopic diseases

Persistence of certain infectious agents, especially bacteria within intracellular compartments of APCs and certain extracellular parasites, can cause destructive induration with granuloma formation and giant cells, termed the *granulomatous form of DH.* Also, immune complex deposition and innate immune mechanisms in response to heavy metal or foreign-body reactions can cause granulomatous inflammation, in which the inflammatory cascade (resulting in DH) is triggered in the absence of specific T lymphocytes. Unfortunately, for most clinical entities in which T-lymphocyte responses are suspected, especially autoimmune disorders such as multiple sclerosis or rheumatoid arthritis, the precise immunologic mechanisms remain highly speculative. See Clinical Example 2-3.

CLINICAL EXAMPLE 2-3

***Toxocara* granuloma (Th2 DH)** *Toxocara canis* is a nematode parasite that infects up to 2% of all children worldwide. *T canis* infection occasionally produces inflammatory vitreoretinal manifestations (see Chapter 12). Humans become infected through ingestion of viable *T canis* eggs, which subsequently mature into larvae within the intestine. Animal models and immunopathogenesis of human nematode infections at other sites suggest that the primary immune response begins in the gut. The primary processing phase produces a strong Th2 response, leading to a primary effector response with production of IgM, IgG, and IgE antibodies, as well as DH T lymphocytes. Through immune evasion, a few larvae may disseminate hematogenously to the choroid or retina and subsequently invade the retina and/or vitreous. There, a Th2-mediated T-lymphocyte effector response against larva antigens releases Th2 cytokines to induce eosinophil and macrophage infiltration, causing the characteristic eosinophilic granuloma seen in the eye. In addition, antilarval B lymphocytes can infiltrate the eye and are induced to secrete various immunoglobulins, especially IgE. Finally, eosinophils, in part by attachment through Fc receptors, can recognize IgE or IgG bound to parasites and release cytotoxic granules containing the antiparasitic cationic protein directly near the larvae, using a mechanism similar to antibody-dependent cellular cytotoxicity.

Yasuda K, Nakanishi K. Host responses to intestinal nematodes. *Int Immunol.* 2018;30(3):93–102. doi:https://doi.org/10.1093/intimm/dxy002

Sympathetic ophthalmia (Th1 DH) Sympathetic ophthalmia is a bilateral granulomatous panuveitis that develops following penetrating trauma to an eye (see Chapter 10). This disorder represents one of the few human diseases in which autoimmunity can be directly linked to an initiating event. In most cases, penetrating injury activates the afferent phase of the immune response. The precise pathogenesis of sympathetic ophthalmia is unclear, but a leading hypothesis is that the injury causes (1) a de novo primary immunization to autoantigens—perhaps because externalization of previously sequestered uveal antigens through the wound initiates an immune response in the conjunctiva or extraocular sites—and (2) a change in the immunologic microenvironment of the retina, retinal pigment epithelium, and uvea to allow an immune response within the eye to overcome local suppressor mechanisms.

The inflammatory effector response is generally thought to be dominated by a Th1-mediated DH mechanism generated in response to uveal or retinal antigens, with $CD4^+$ T-lymphocyte predominance early in the disease course. Activated macrophages are also numerous in granulomas, and Th1 cytokines have been identified in the vitreous of affected patients. Th1-mediated DH effector mechanisms also are implicated in many other forms of ocular inflammation (see Table 2-2).

Boyd SR, Young S, Lightman S. Immunopathology of the noninfectious posterior and intermediate uveitides. *Surv Ophthalmol.* 2001;46(3):209–233.

Cytotoxic lymphocytes

Cytotoxic T lymphocytes Cytotoxic T lymphocytes (CTLs) are a subset of antigen-specific T lymphocytes bearing the CD8 marker that are especially good at killing tumor cells and virus-infected cells. CTLs can also mediate graft rejection and some types of autoimmunity. In most cases, the ideal antigen for CTLs is an intracellular protein that occurs naturally or is produced because of viral infection. CTLs appear to require assistance from $CD4^+$ helper T-cell signals in order to fully differentiate. Also, local $CD4^+$ T lymphocytes and other accessory costimulatory molecules on the target cell may be required to achieve maximal killing.

CTLs kill cells in 1 of 2 ways: "assassination" or "suicide" induction (Fig 2-7). *Assassination* refers to CTL-mediated lysis of target cells. A specialized pore-forming protein called *perforin* is released that inserts into cell membranes and causes osmotic lysis of the cell. *Suicide induction* refers to the capability of CTLs to stimulate programmed cell death of target cells, called *apoptosis,* using the CD95 ligand (FasL) to activate the CD95 receptor (Fas) on target cells. Alternatively, CTLs can release cytokines such as TNF to induce apoptosis. CTLs produce low-grade lymphocytic infiltrates within tumors or infected tissues and usually kill without causing significant inflammation.

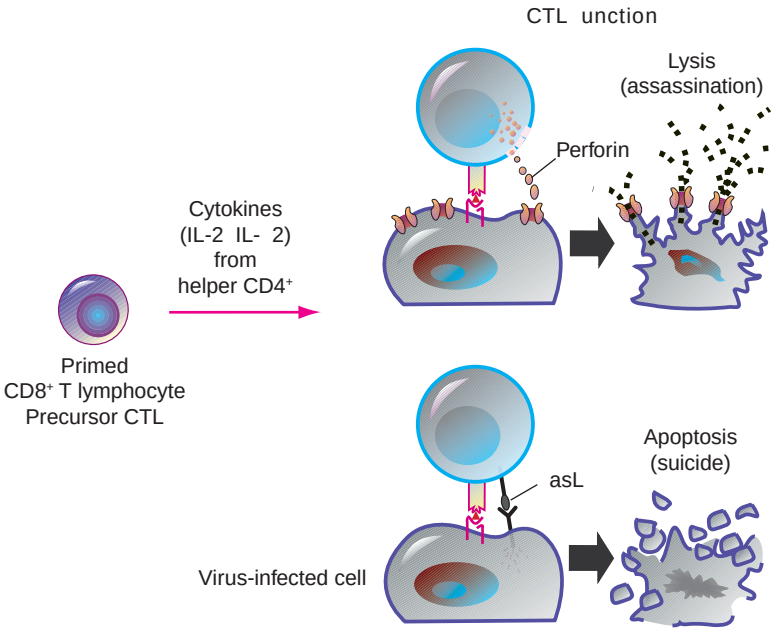

Figure 2-7 Schematic representation of the 2 major mechanisms of $CD8^+$ T-lymphocyte cytotoxicity. $CD8^+$ T lymphocytes, having undergone initial priming in the lymph node, enter the tissue site, where they again encounter antigen in the form of infected target cells. Upon restimulation, usually requiring $CD4^+$ T-cell factors, $CD8^+$ T lymphocytes are activated, fully becoming CTLs. CTLs can kill by lysing the infected cell using a pore-forming protein called *perforin* or by inducing programmed cell death, called *apoptosis,* using either Fas ligand (FasL) or cytokine-mediated mechanisms. *(Illustration by Barb Cousins, modified by Joyce Zavarro.)*

Natural killer cells Natural killer (NK) cells are a subset of non-T, non-B lymphocytes. They kill tumor cells and virus-infected cells using the same molecular mechanisms as CTLs. Unlike CTLs, NK cells do not have a specific antigen receptor and do not require priming or prior activation. Instead, they express multiple activating and inhibitory receptors; the balance of signals from these receptors determines whether NK cells are activated or inhibited. Activating receptors trigger the NK cell to kill target cells that are missing HLA class I molecules or those that display inappropriate molecules. Inhibitory NK receptors prevent NK cells from indiscriminately attacking healthy host tissue by recognizing ligands that ought to be present.

Combined Antibody and Cellular Effector Mechanisms

Antibody-dependent cellular cytotoxicity

An antibody can bind to a cell-associated antigen such as a tumor or viral antigen, but if the antibody is not a member of a subclass that activates complement, the antibody may not directly induce cytotoxicity. However, various leukocytes may recognize the exposed Fc domain of the bound antibody and then activate various leukocyte cytotoxic mechanisms, including degranulation and cytokine production. Classically, *antibody-dependent cellular cytotoxicity (ADCC)* was observed to be mediated by a subset of large granular (non-T, non-B) lymphocytes, called *killer cells,* that induce cell death in a manner similar to CTLs. The killer cell itself is nonspecific but gains antigen specificity through interaction with specific antibody. Macrophages, NK cells, certain T lymphocytes, and neutrophils can also participate in ADCC using other Fc receptor types. An IgE-dependent form of ADCC may also exist for eosinophils.

ADCC is presumed to be important in tumor immune surveillance, antimicrobial host protection, graft rejection, and certain autoimmune diseases, such as cutaneous systemic lupus erythematosus. However, this effector mechanism probably does not play an important role in uveitis, although it might contribute to antiparasitic immunity.

Acute IgE-mediated mast cell degranulation

Mast cells bind IgE antibodies to their surface through a high-affinity Fc receptor specific for IgE molecules, positioning the antigen-recognition site of the bound IgE externally. Combining 2 adjacent IgE antibody molecules with a specific allergen causes degranulation of the mast cell and release of preformed and de novo synthesized mediators within minutes. This acute inflammatory reaction is called *immediate hypersensitivity* (previously called *Gell and Coombs type I,* or *anaphylaxis*).

Preformed mediators include histamine, serotonin, proteoglycans (heparin), neutral proteases (ie, tryptase, chymase), chemotactic factors (eosinophil, neutrophil, or monocyte), and possibly basic fibroblast growth factor. Among the newly generated mediators are the arachidonic acid metabolites prostaglandin D_2, leukotrienes, and thromboxane as well as Th2 cytokines (IL-4, IL-5, IL-13), TNF-α, IL-1, and CCL2.

Releasing these mediators results in vasodilation, increased capillary permeability, contraction of bronchial and gastrointestinal smooth muscle, and increased mucous secretion in mucosal sites. Mast cell–derived cytokines play a role in the late phase of allergic response by activating endothelial cells to recruit eosinophils and other inflammatory cells to the site

of hypersensitivity reactions, thus sustaining inflammation. When severe, the immediate hypersensitivity response can produce a systemic reaction, with manifestations ranging from generalized skin lesions, such as erythema, urticaria, or angioedema, to severely altered vascular permeability with plasma leakage into tissues and airway obstruction or hypotensive shock. The use of antihistamine medications such as diphenhydramine to treat acute allergic reactions illustrates the central role of histamine in immediate hypersensitivity reactions.