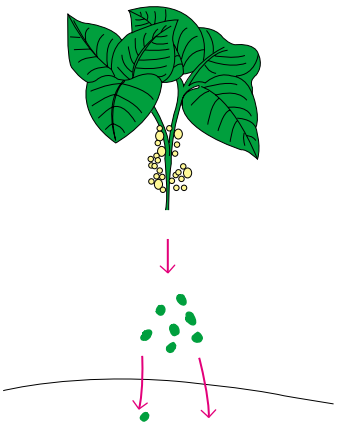
chapter 16

Hypersensitive Reactions



A Second Exposure to Poison Oak May Result in Delayed-Type Hypersensitivity

An immune response mobilizes a battery of effector molecules that act to remove antigen by various mechanisms described in previous chapters. Generally, these effector molecules induce a localized inflammatory response that eliminates antigen without extensively damaging the host's tissue. Under certain circumstances, however, this inflammatory response can have deleterious effects, resulting in significant tissue damage or even death. This inappropriate immune response is termed **hypersensitivity** or **allergy.** Although the word *hypersensitivity* implies an increased response, the response is not always heightened but may, instead, be an inappropriate immune response to an antigen. Hypersensitive reactions may develop in the course of either humoral or cell-mediated responses.

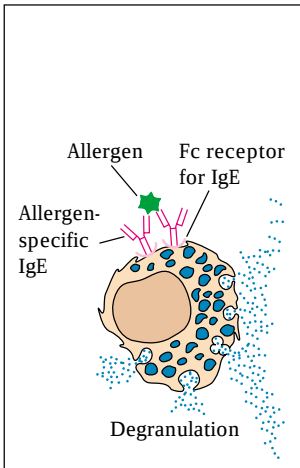
The ability of the immune system to respond inappropriately to antigenic challenge was recognized early in this century. Two French scientists, Paul Portier and Charles Richet, investigated the problem of bathers in the Mediterranean reacting violently to the stings of Portuguese Man of War jellyfish. Portier and Richet concluded that the localized reaction of the bathers was the result of toxins. To counteract this reaction, the scientists experimented with the use of isolated jellyfish toxins as vaccines. Their first attempts met with disastrous results. Portier and Richet injected dogs with the purified toxins, followed later by a booster of toxins. Instead of reacting to the booster by producing antibodies against the toxins, the dogs immediately reacted with vomiting, diarrhea, asphyxia, and, in some instances, death. Clearly this was an instance where the animals "overreacted" to the antigen. Portier and Richet coined the term *anaphylaxis,* loosely translated from Greek to mean the opposite of *prophylaxis,* to describe this overreaction. Richet was subsequently awarded the Nobel Prize in Physiology or Medicine in 1913 for his work on anaphylaxis.

We currently refer to anaphylactic reactions within the humoral branch initiated by antibody or antigen-antibody complexes as **immediate hypersensitivity,** because the symptoms are manifest within minutes or hours after a sensitized recipient encounters antigen. **Delayed-type hypersensitivity (DTH)** is so named in recognition of the delay of symptoms until days after exposure. This chapter examines the mechanisms and consequences of the four primary types of hypersensitive reactions.

Gell and Coombs Classification

Several forms of hypersensitive reaction can be distinguished, reflecting differences in the effector molecules generated in the course of the reaction. In immediate hypersensitive reactions, different antibody isotypes induce different immune effector molecules. IgE antibodies, for example, induce mast-cell degranulation with release of histamine and other biologically active molecules. IgG and IgM antibodies, on the other hand, induce hypersensitive reactions by activating complement. The effector molecules in the complement reactions are the membrane-attack complex and such complement split products as C3a, C4a, and C5a. In delayed-type hypersensitivity reactions, the effector molecules are various cytokines secreted by activated T_H or T_C cells.

VISUALIZING CONCEPTS

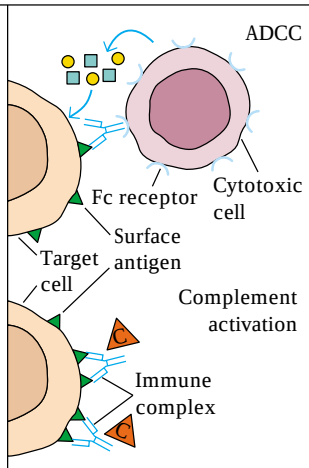


Type I

IgE-Mediated Hypersensitivity

Ag induces crosslinking of IgE bound to mast cells and basophils with release of vasoactive mediators

Typical manifestations include systemic anaphylaxis and localized anaphylaxis such as hay fever, asthma, hives, food allergies, and eczema

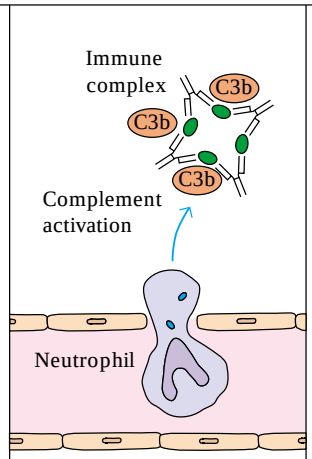


Type II

IgG-Mediated Cytotoxic Hypersensitivity

Ab directed against cell surface antigens meditates cell destruction via complement activation or ADCC

Typical manifestations include blood transfusion reactions, erythroblastosis fetalis, and autoimmune hemolytic anemia

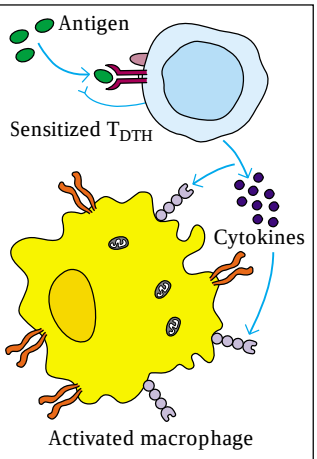


Type III

Immune Complex-Mediated Hypersensitivity

Ag-Ab complexes deposited in various tissues induce complement activation and an ensuing inflammatory response mediated by massive infiltration of neutrophils

Typical manifestations include localized Arthus reaction and generalized reactions such as serum sickness, necrotizing vasculitis, glomerulnephritis, rheumatoid arthritis, and systemic lupus erythematosus



Type IV

Cell-Mediated Hypersensitivity

Sensitized T_H1 cells release cytokines that activate macrophages or T_C cells which mediate direct cellular damage

Typical manifestations include contact dermatitis, tubercular lesions and graft rejection

FIGURE 16-1 The four types of hypersensitive responses.

As it became clear that several different immune mechanisms give rise to hypersensitive reactions, P. G. H. Gell and R. R. A. Coombs proposed a classification scheme in which hypersensitive reactions are divided into four types. Three types of hypersensitivity occur within the humoral branch and are mediated by antibody or antigen-antibody complexes: IgE-mediated (type I), antibody-mediated (type II), and immune complex–mediated (type III). A fourth type of hypersensitivity depends on reactions within the cell-mediated branch, and is termed delayed-type hypersensitivity, or DTH (type IV). Each type involves distinct mechanisms, cells, and mediator molecules (Figure 16-1). This classification scheme has served an important function in identifying the mechanistic differences among various hypersensitive reactions, but it is important to point out that secondary effects blur the boundaries between the four categories.

IgE-Mediated (Type I) Hypersensitivity

A type I hypersensitive reaction is induced by certain types of antigens referred to as **allergens,** and has all the hallmarks of a normal humoral response. That is, an allergen induces a humoral antibody response by the same mechanisms as described in Chapter 11 for other soluble antigens, resulting in the generation of antibody-secreting plasma cells and memory cells. What distinguishes a type I hypersensitive response from a normal humoral response is that the plasma

cells secrete IgE. This class of antibody binds with high affinity to **Fc receptors** on the surface of tissue mast cells and blood basophils. Mast cells and basophils coated by IgE are said to be sensitized. A later exposure to the same allergen cross-links the membrane-bound IgE on sensitized mast cells and basophils, causing **degranulation** of these cells (Figure 16-2). The pharmacologically active mediators released from the granules act on the surrounding tissues. The principal effects—vasodilation and smooth-muscle contraction—may be either systemic or localized, depending on the extent of mediator release.

There Are Several Components of Type I Reactions

As depicted in Figure 16-2, several components are critical to development of type I hypersensitive reactions. This section will consider these components first and then describe the mechanism of degranulation.

ALLERGENS

The majority of humans mount significant IgE responses only as a defense against parasitic infections. After an individual has been exposed to a parasite, serum IgE levels increase and remain high until the parasite is successfully cleared from the body. Some persons, however, may have an abnormality called **atopy,** a hereditary predisposition to the development of immediate hypersensitivity reactions against common environmental antigens. The IgE regulatory defects suffered by atopic individuals allow nonparasitic antigens to stimulate inappropriate IgE production, leading to tissue-damaging type I hypersensitivity. The term *allergen* refers specifically to nonparasitic antigens capable of stimulating type I hypersensitive responses in allergic individuals.

The abnormal IgE response of atopic individuals is at least partly genetic—it often runs in families. Atopic individuals have abnormally high levels of circulating IgE and also more than normal numbers of circulating eosinophils. These individuals are more susceptible to allergies such as hay fever, eczema, and asthma. The genetic propensity to atopic responses has been mapped to several candidate loci. One locus, on chromosome 5q, is linked to a region that encodes a variety of cytokines, including IL-3, IL-4, IL-5, IL-9, IL-13, and GM-CSF. A second locus, on chromosome 11q, is linked to a region that encodes the β chain of the high-affinity IgE receptor. It is known that inherited atopy is multigenic and that other loci probably also are involved. Indeed, as information from the Human Genome Project is analyzed, other candidate genes may be revealed.

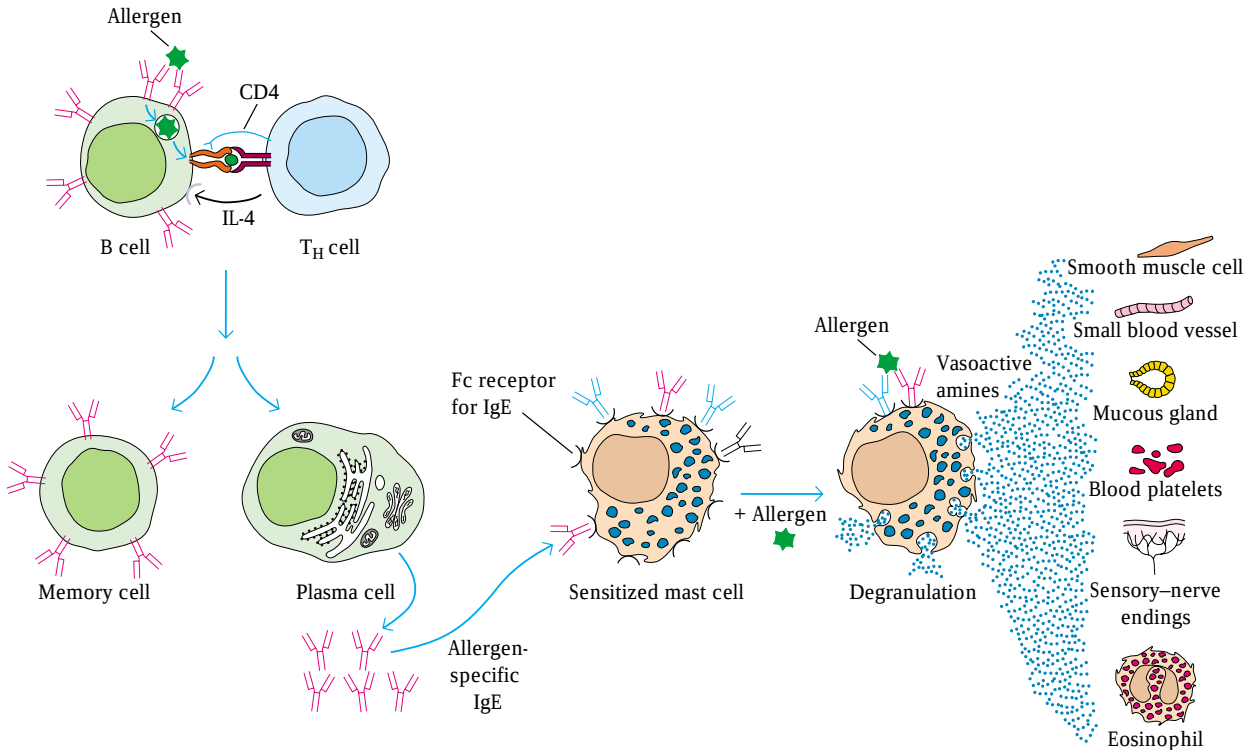


FIGURE 16-2 General mechanism underlying a type I hypersensitive reaction. Exposure to an allergen activates B cells to form IgE-secreting plasma cells. The secreted IgE molecules bind to IgE-specific Fc receptors on mast cells and blood basophils. (Many molecules of IgE with various specificities can bind to the IgE-Fc receptor.) Second exposure to the allergen leads to crosslinking of the bound IgE, triggering the release of pharmacologically active mediators, vasoactive amines, from mast cells and basophils. The mediators cause smooth-muscle contraction, increased vascular permeability, and vasodilation.

TABLE 16-1 Common allergens associated with type I hypersensitivity

Proteins	Foods
Foreign serum	Nuts
Vaccines	Seafood
	Eggs
Plant pollens	Peas, beans
Rye grass	Milk
Ragweed	
Timothy grass	Insect products
Birch trees	Bee venom
	Wasp venom
Drugs	Ant venom
Penicillin	Cockroach calyx
Sulfonamides	Dust mites
Local anesthetics	
Salicylates	Mold spores
	Animal hair and dander

Most allergic IgE responses occur on mucous membrane surfaces in response to allergens that enter the body by either inhalation or ingestion. Of the common allergens listed in Table 16-1, few have been purified and characterized. Those that have include the allergens from rye grass pollen, ragweed pollen, codfish, birch pollen, timothy grass pollen, and bee venom. Each of these allergens has been shown to be a multiantigenic system that contains a number of allergenic components. Ragweed pollen, a major allergen in the United States, is a case in point. It has been reported that a square mile of ragweed yields 16 tons of pollen in a single season. Indeed, all regions of the United States are plagued by ragweed pollen as well as pollen from trees indigenous to the region. The pollen particles are inhaled, and their tough outer wall is dissolved by enzymes in the mucous secretions, releasing the allergenic substances. Chemical fractionation of ragweed has revealed a variety of substances, most of which are not allergenic but are capable of eliciting an IgM or IgG response. Of the five fractions that are allergenic (i.e., able to induce an IgE response), two evoke allergenic reactions in about 95% of ragweed-sensitive individuals and are called major allergens; these are designated the E and K fractions. The other three, called Ra3, Ra4, and Ra5, are minor allergens that induce an allergic response in only 20% to 30% of sensitive subjects.

Why are some pollens (e.g., ragweed) highly allergenic, whereas other equally abundant pollens (e.g., nettle) are rarely allergenic? No single physicochemical property seems to distinguish the highly allergenic E and K fractions of ragweed from the less allergenic Ra3, Ra4, and Ra5 fractions and from the nonallergenic fractions. Rather, allergens as a group appear to possess diverse properties. Some allergens, including foreign serum and egg albumin, are potent antigens; others, such as plant pollens, are weak antigens. Although most allergens are small proteins or protein-bound substances having a molecular weight between 15,000 and 40,000, attempts to identify some common chemical property of these antigens have failed. It appears that allergenicity is a consequence of a complex series of interactions involving not only the allergen but also the dose, the sensitizing route, sometimes an adjuvant, and—most important, as noted above—the genetic constitution of the recipient.

REAGINIC ANTIBODY (IgE)

As described in Chapter 4, the existence of a human serum factor that reacts with allergens was first demonstrated by K. Prausnitz and H. Kustner in 1921. The local wheal and flare response that occurs when an allergen is injected into a sensitized individual is called the P-K reaction. Because the serum components responsible for the P-K reaction displayed specificity for allergen, they were assumed to be antibodies, but the nature of these P-K antibodies, or **reagins,** was not demonstrated for many years.

Experiments conducted by K. and T. Ishizaka in the mid-1960s showed that the biological activity of reaginic antibody in a P-K test could be neutralized by rabbit antiserum against whole atopic human sera but not by rabbit antiserum specific for the four human immunoglobulin classes known at that time (IgA, IgG, IgM, and IgD) (Table 16-2). In addition, when rabbits were immunized with sera from ragweed-sensitive individuals, the rabbit antiserum could inhibit (neutralize) a positive ragweed P-K test even after precipitation of the rabbit antibodies specific for the human IgG, IgA, IgM, and IgD isotypes. The Ishizakas called this new isotype IgE in reference to the E antigen of ragweed that they used to characterize it.

Serum IgE levels in normal individuals fall within the range of 0.1–0.4 μg/ml; even the most severely allergic individuals rarely have IgE levels greater than 1 μg/ml. These low levels made physiochemical studies of IgE difficult; it was not until the discovery of an IgE myeloma by S. G. O. Johansson and H. Bennich in 1967 that extensive chemical analysis of IgE could be undertaken. IgE was found to be composed of two heavy ε and two light chains with a combined molecular weight of 190,000. The higher molecular weight as compared with IgG (150,000) is due to the presence of an additional constant-region domain (see Figure 4-13). This additional domain (C_H4) contributes to an altered conformation of the Fc portion of the molecule that enables it to bind to glycoprotein receptors on the surface of basophils and mast cells. Although the half-life of IgE in the serum is only 2–3 days, once IgE has been bound to its receptor on mast cells and basophils, it is stable in that state for a number of weeks.

MAST CELLS AND BASOPHILS

The cells that bind IgE were identified by incubating human leukocytes and tissue cells with either ^{125}I-labeled IgE myeloma protein or ^{125}I-labeled anti-IgE. In both cases, autoradiography revealed that the labeled probe bound with high affinity to blood basophils and tissue mast cells. Basophils are

TABLE 16-2 Identification of IgE based on reactivity of atopic serum in P-K test

Serum	Treatment	Allergen added	P-K reaction at skin site
Atopic	None	–	–
Atopic	None	+	+
Nonatopic	None	+	–
Atopic	Rabbit antiserum to human atopic serum*	+	–
Atopic	Rabbit antiserum to human IgM, IgG, IgA, and IgD†	+	+

*Serum from an atopic individual was injected into rabbits to produce antiserum against human atopic serum. When this antiserum was reacted with human atopic serum, it neutralized the P-K reaction.

†Serum from an atopic individual was reacted with rabbit antiserum to the known classes of human antibody (IgM, IgA, IgG, and IgD) to remove these isotypes from the atopic serum. The treated atopic serum continued to give a positive P-K reaction, indicating that a new immunoglobulin isotype was responsible for this reactivity.

SOURCE: Based on K. Ishizaka and T. Ishizaka, 1967, *J. Immunol.* **99**:1187.

granulocytes that circulate in the blood of most vertebrates; in humans, they account for 0.5%–1.0% of the circulating white blood cells. Their granulated cytoplasm stains with basic dyes, hence the name basophil. Electron microscopy reveals a multilobed nucleus, few mitochondria, numerous glycogen granules, and electron-dense membrane-bound granules scattered throughout the cytoplasm that contain pharmacologically active mediators (see Figure 2-10c).

Mast-cell precursors are formed in the bone marrow during hematopoiesis and are carried to virtually all vascularized peripheral tissues, where they differentiate into mature cells. Mast cells are found throughout connective tissue, particularly near blood and lymphatic vessels. Some tissues, including the skin and mucous membrane surfaces of the respiratory and gastrointestinal tracts, contain high concentrations of mast cells; skin, for example, contains 10,000 mast cells per mm^3. Electron micrographs of mast cells reveal numerous membrane-bounded granules distributed throughout the cytoplasm, which, like those in basophils, contain pharmacologically active mediators (Figure 16-3). After activation, these mediators are released from the granules, resulting in the clinical manifestations of the type I hypersensitive reaction.

Mast cell populations in different anatomic sites differ significantly in the types and amounts of allergic mediators they contain and in their sensitivity to activating stimuli and cytokines. Mast cells also secrete a large variety of cytokines that affect a broad spectrum of physiologic, immunologic, and pathologic processes (see Table 12-1).

IgE-BINDING Fc RECEPTORS

The reaginic activity of IgE depends on its ability to bind to a receptor specific for the Fc region of the ϵ heavy chain. Two

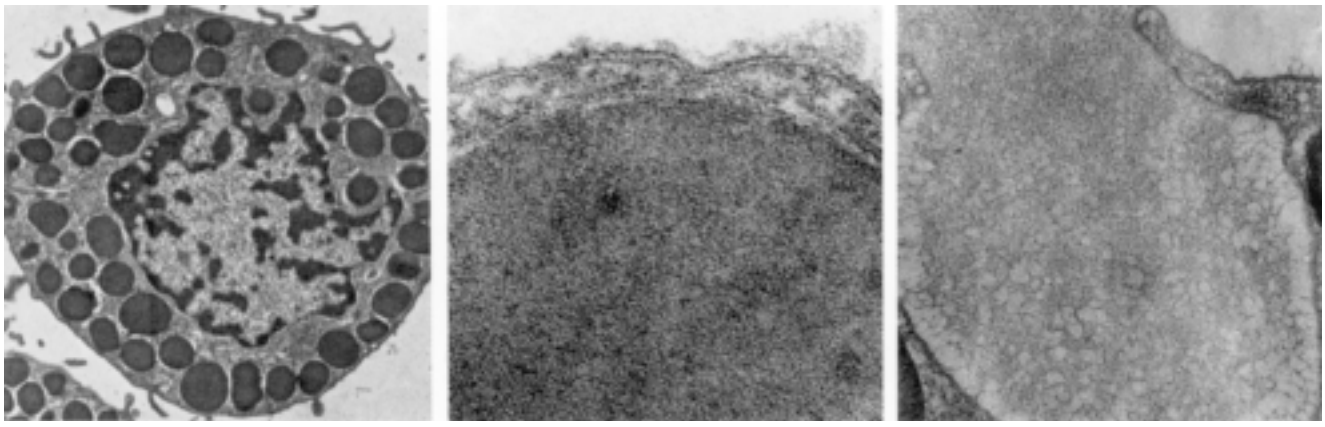


FIGURE 16-3 (a) Electron micrograph of a typical mast cell reveals numerous electron-dense membrane-bounded granules prior to degranulation. (b) Close-up of intact granule underlying the plasma membrane of a mast cell. (c) Granule releasing its contents (towards top left) during degranulation. *[From S. Burwen and B. Satir, 1977, J. Cell Biol. **73**:662.]*

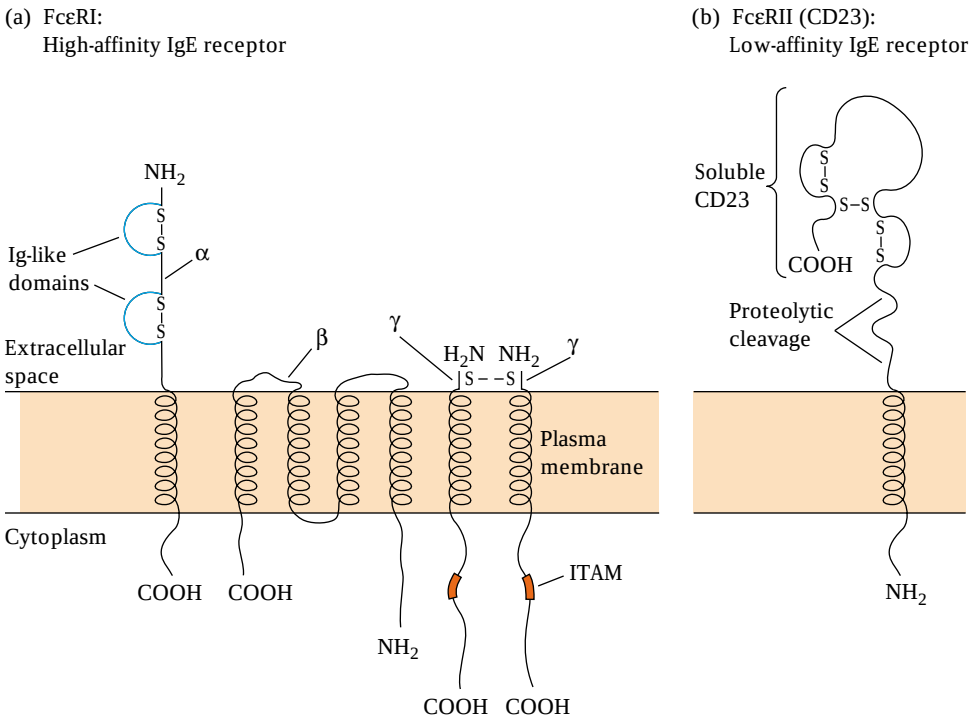


FIGURE 16-4 Schematic diagrams of the high-affinity FcεRI and low-affinity FcεRII receptors that bind the Fc region of IgE. (a) Each γ chain of the high-affinity receptor contains an ITAM, a motif also present in the Ig-α/Ig-β heterodimer of the B-cell receptor and in the CD3 complex of the T-cell receptor. (b) The low-affinity receptor is unusual because it is oriented in the membrane with its NH_2-terminus directed toward the cell interior and its COOH-terminus directed toward the extracellular space.

classes of FcεR been identified, designated FcεRI and FcεRII, which are expressed by different cell types and differ by 1000-fold in their affinity for IgE.

HIGH-AFFINITY RECEPTOR (FCεRI) Mast cells and basophils express FcεRI, which binds IgE with a high affinity (K_D = 1–2 × 10^{-9} M). The high affinity of this receptor enables it to bind IgE despite the low serum concentration of IgE (1 × 10^{-7}). Between 40,000 and 90,000 FcεRI molecules have been shown to be present on a human basophil.

The FcεRI receptor contains four polypeptide chains: an α and a β chain and two identical disulfide-linked γ chains (Figure 16-4a). The external region of the α chain contains two domains of 90 amino acids that are homologous with the immunoglobulin-fold structure, placing the molecule in the immunoglobulin superfamily (see Figure 4-19). FcεRI interacts with the C_H3/C_H3 and C_H4/C_H4 domains of the IgE molecule via the two Ig-like domains of the α chain. The β chain spans the plasma membrane four times and is thought to link the α chain to the γ homodimer. The disulfide-linked γ chains extend a considerable distance into the cytoplasm. Each γ chain has a conserved sequence in its cytosolic domain known as an immunoreceptor tyrosine-based activation motif (ITAM). As described earlier, two other membrane receptors that have this motif are CD3 and the associated ζ chains of the T-cell receptor complex (see Figure 10-10) and the Ig-α/Ig-β chains associated with membrane immunoglobulin on B cells (see Figure 11-7). The ITAM motif on these three receptors interacts with protein tyrosine kinases to transduce an activating signal to the cell. Allergen-mediated crosslinkage of the bound IgE results in aggregation of the FcεRI receptors and rapid tyrosine phosphorylation, which initiates the process of mast-cell degranulation. The role of FcεRI in anaphylaxis is confirmed by experiments conducted in mice that lack FcεRI. These mice have normal levels of mast cells but are resistant to localized and systemic anaphylaxis.

LOW-AFFINITY RECEPTOR (FCεRII) The other IgE receptor, designated FcεRII (or CD23), is specific for the C_H3/C_H3 domain of IgE and has a lower affinity for IgE (K_D = 1 × 10^{-6}M) than does FcεRI (Figure 16-4b). The FcεRII receptor appears to play a variety of roles in regulating the intensity of the IgE response. Allergen crosslinkage of IgE bound to FcεRII has been shown to activate B cells, alveolar macrophages, and eosinophils. When this receptor is blocked with monoclonal antibodies, IgE secretion by B cells is diminished. A soluble form of FcεRII (or sCD23), which is

generated by autoproteolysis of the membrane receptor, has been shown to enhance IgE production by B cells. Interestingly, atopic individuals have higher levels of CD23 on their lymphocytes and macrophages and higher levels of sCD23 in their serum than do nonatopic individuals.

IgE Crosslinkage Initiates Degranulation

The biochemical events that mediate degranulation of mast cells and blood basophils have many features in common. For simplicity, this section presents a general overview of mast-cell degranulation mechanisms without calling attention to the slight differences between mast cells and basophils. Although mast-cell degranulation generally is initiated by allergen crosslinkage of bound IgE, a number of other stimuli can also initiate the process, including the anaphylatoxins (C3a, C4a, and C5a) and various drugs. This section focuses on the biochemical events that follow allergen crosslinkage of bound IgE.

RECEPTOR CROSSLINKAGE

IgE-mediated degranulation begins when an allergen crosslinks IgE that is bound (fixed) to the Fc receptor on the surface of a mast cell or basophil. In itself, the binding of IgE to FcεRI apparently has no effect on a target cell. It is only after allergen crosslinks the fixed IgE-receptor complex that degranulation proceeds. The importance of crosslinkage is indicated by the inability of monovalent allergens, which cannot crosslink the fixed IgE, to trigger degranulation.

Experiments have revealed that the essential step in degranulation is crosslinkage of two or more FcεRI molecules—with or without bound IgE. Although crosslinkage is normally effected by the interaction of fixed IgE with divalent or multivalent allergen, it also can be effected by a variety of experimental means that bypass the need for allergen and in some cases even for IgE (Figure 16-5).

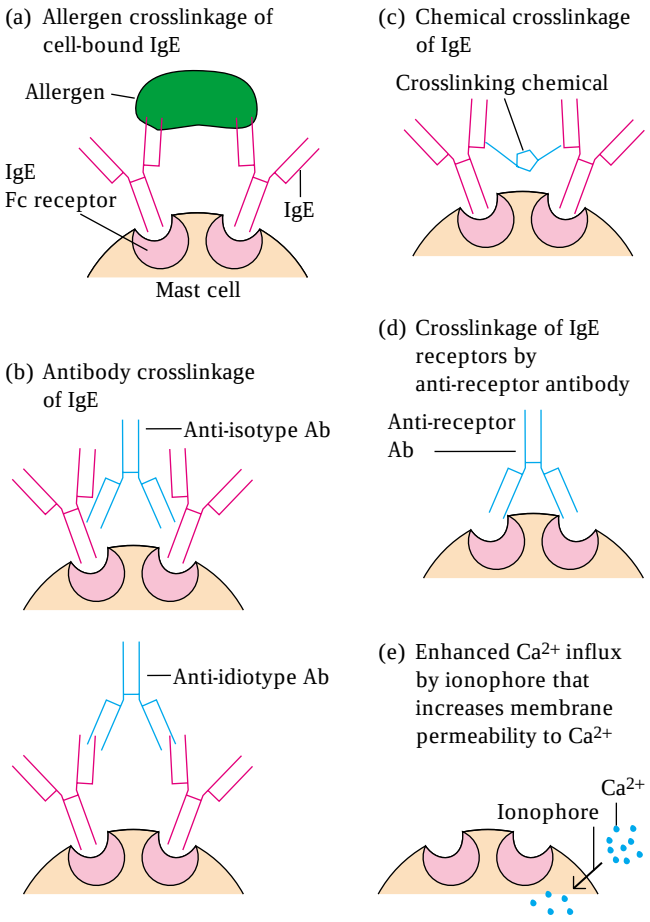


FIGURE 16-5 Schematic diagrams of mechanisms that can trigger degranulation of mast cells. Note that mechanisms (b) and (c) do not require allergen; mechanisms (d) and (e) require neither allergen nor IgE; and mechanism (e) does not even require receptor crosslinkage.

Intracellular Events Also Regulate Mast-Cell Degranulation

The cytoplasmic domains of the β and γ chains of FcεRI are associated with protein tyrosine kinases (PTKs). Crosslinkage of the FcεRI receptors activates the associated PTKs, resulting in the phosphorylation of tyrosines within the ITAMs of the γ subunit as well as phosphorylation of residues on the β subunit and on phospholipase C. These phosphorylation events induce the production of a number of second messengers that mediate the process of degranulation (Figure 16-6).

Within 15 s after crosslinkage of FcεRI, methylation of various membrane phospholipids is observed, resulting in an increase in membrane fluidity and the formation of Ca^{2+} channels. An increase of Ca^{2+} reaches a peak within 2 min of FcεRI crosslinkage (Figure 16-7). This increase is due both to the uptake of extracellular Ca^{2+} and to a release of Ca^{2+} from intracellular stores in the endoplasmic reticulum (see Figure 16-6). The Ca^{2+} increase eventually leads to the formation of arachidonic acid, which is converted into two classes of potent mediators: **prostaglandins** and **leukotrienes** (see Figure 16-6). The increase of Ca^{2+} also promotes the assembly of microtubules and the contraction of microfilaments, both of which are necessary for the movement of granules to the plasma membrane. The importance of the Ca^{2+} increase in mast-cell degranulation is highlighted by the use of drugs, such as disodium cromoglycate (cromolyn sodium), that block this influx as a treatment for allergies.

Concomitant with phospholipid methylation and Ca^{2+} increase, there is a transient increase in the activity of membrane-bound adenylate cyclase, with a rapid peak of its reaction product, cyclic adenosine monophosphate (cAMP), reached about 1 min after crosslinkage of FcεRI (see Figure 16-7). The effects of cAMP are exerted through the activation of cAMP-dependent

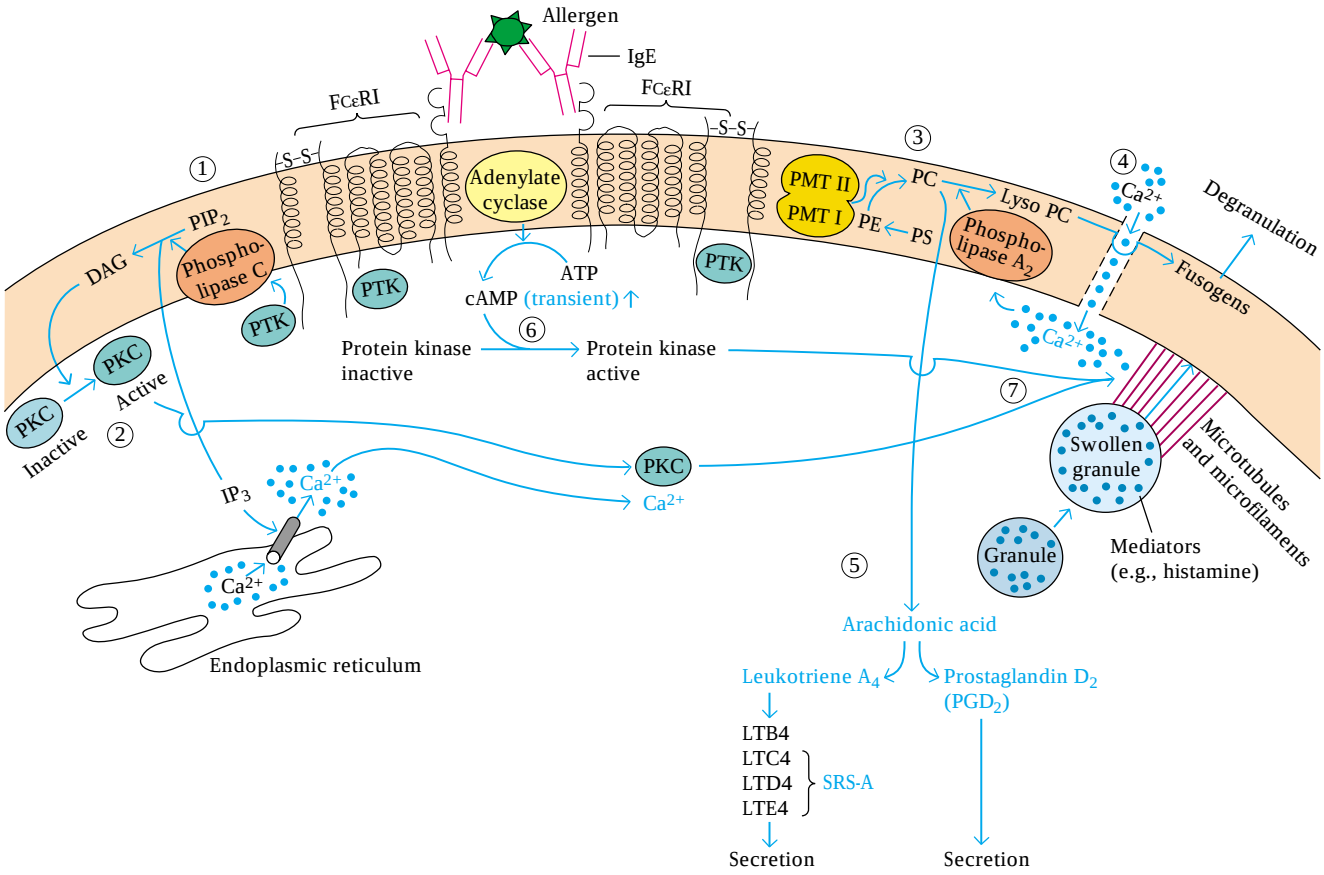


FIGURE 16-6 Diagrammatic overview of biochemical events in mast-cell activation and degranulation. Allergen crosslinkage of bound IgE results in FcεRI aggregation and activation of protein tyrosine kinase (PTK). (1) PTK then phosphorylates phospholipase C, which converts phosphatidylinositol-4,5 bisphosphate (PIP_2) into diacylglycerol (DAG) and inositol triphosphate (IP_3). (2) DAG activates protein kinase C (PKC), which with Ca^{2+} is necessary for microtubular assembly and the fusion of the granules with the plasma membrane. IP_3 is a potent mobilizer of intracellular Ca^{2+} stores. (3) Crosslinkage of FcεRI also activates an enzyme that converts phosphatidylserine (PS) into phosphatidylethanolamine (PE). Eventually, PE is methylated to form phosphatidylcholine (PC) by the phospholipid methyl transferase enzymes I and II (PMT I and II). (4) The accumulation of PC on the exterior surface of the plasma membrane causes an increase in membrane fluidity and facilitates the formation of Ca^{2+} channels. The resulting influx of Ca^{2+} activates phospholipase A_2, which promotes the breakdown of PC into lysophosphatidylcholine (lyso PC) and arachidonic acid. (5) Arachidonic acid is converted into potent mediators: the leukotrienes and prostaglandin D_2. (6) FcεRI crosslinkage also activates the membrane adenylate cyclase, leading to a transient increase of cAMP within 15 s. A later drop in cAMP levels is mediated by protein kinase and is required for degranulation to proceed. (7) cAMP-dependent protein kinases are thought to phosphorylate the granule-membrane proteins, thereby changing the permeability of the granules to water and Ca^{2+}. The consequent swelling of the granules facilitates fusion with the plasma membrane and release of the mediators.

protein kinases, which phosphorylate proteins on the granule membrane, thereby changing the permeability of the granules to water and Ca^{2+} (see Figure 16-6). The consequent swelling of the granules facilitates their fusion with the plasma membrane, releasing their contents. The increase in cAMP is transient and is followed by a drop in cAMP to levels below baseline (see Figure 16-7). This drop in cAMP appears to be necessary for degranulation to proceed; when cAMP levels are increased by certain drugs, the degranulation process is blocked. Several of these drugs are given to treat allergic disorders and are considered later in this section.

Several Pharmacologic Agents Mediate Type I Reactions

The clinical manifestations of type I hypersensitive reactions are related to the biological effects of the mediators released during mast-cell or basophil degranulation. These mediators are pharmacologically active agents that act on local tissues as well as on populations of secondary effector cells, including eosinophils, neutrophils, T lymphocytes, monocytes, and platelets. The mediators thus serve as an amplifying terminal effector mechanism, much as the complement system serves

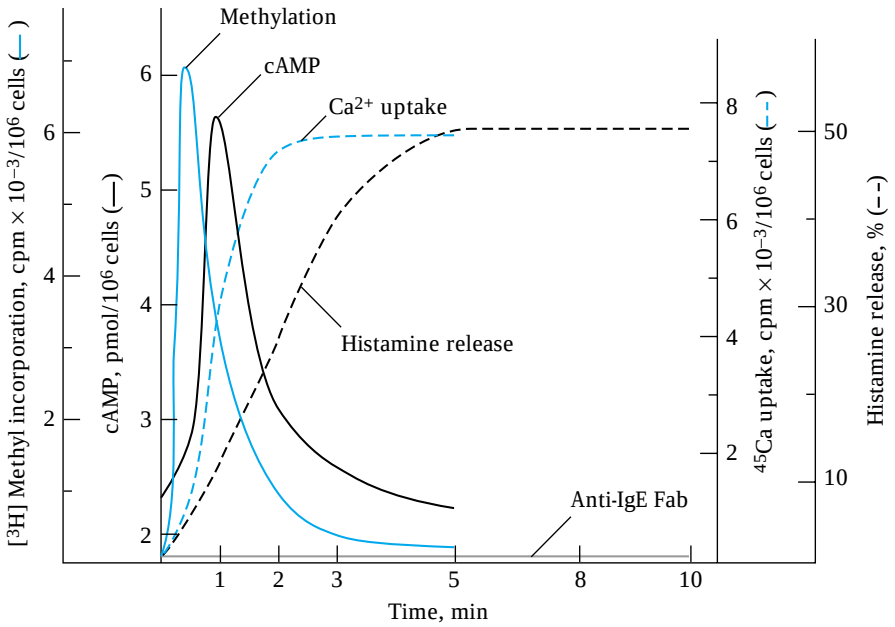


FIGURE 16-7 Kinetics of major biochemical events that follow crosslinkage of bound IgE on cultured human basophils with F(ab′)$_2$ fragments of anti-IgE. Curves are shown for phospholipid methylation (solid blue), cAMP production (solid black), Ca^{2+} influx (dashed blue), and histamine release (dashed black). In control experiments with anti–IgE Fab fragments, no significant changes were observed. *[Adapted from T. Ishizaka et al., 1985,* Int. Arch. Allergy Appl. Immunol. ***77****:137.]*

as an amplifier and effector of an antigen-antibody interaction. When generated in response to parasitic infection, these mediators initiate beneficial defense processes, including vasodilation and increased vascular permeability, which brings an influx of plasma and inflammatory cells to attack the pathogen. On the other hand, mediator release induced by inappropriate antigens, such as allergens, results in unnecessary increases in vascular permeability and inflammation whose detrimental effects far outweigh any beneficial effect.

The mediators can be classified as either primary or secondary (Table 16-3). The primary mediators are produced before degranulation and are stored in the granules. The most significant primary mediators are histamine, proteases, eosinophil chemotactic factor, neutrophil chemotactic factor, and heparin. The secondary mediators either are synthesized after target-cell activation or are released by the breakdown of membrane phospholipids during the degranulation process. The secondary mediators include platelet-activating factor, leukotrienes, prostaglandins, bradykinins, and various cytokines. The differing manifestations of type I hypersensitivity in different species or different tissues partly reflect variations in the primary and secondary mediators present. The main biological effects of several of these mediators are described briefly in the next sections.

HISTAMINE

Histamine, which is formed by decarboxylation of the amino acid histidine, is a major component of mast-cell granules, accounting for about 10% of granule weight. Because it is stored—preformed—in the granules, its biological effects are observed within minutes of mast-cell activation. Once released from mast cells, histamine initially binds to specific receptors on various target cells. Three types of histamine receptors—designated H_1, H_2, and H_3—have been identified; these receptors have different tissue distributions and mediate different effects when they bind histamine.

Most of the biologic effects of histamine in allergic reactions are mediated by the binding of histamine to H_1 receptors. This binding induces contraction of intestinal and bronchial smooth muscles, increased permeability of venules, and increased mucus secretion by goblet cells. Interaction of histamine with H_2 receptors increases vasopermeability and dilation and stimulates exocrine glands. Binding of histamine to H_2 receptors on mast cells and basophils suppresses degranulation; thus, histamine exerts negative feedback on the release of mediators.

LEUKOTRIENES AND PROSTAGLANDINS

As secondary mediators, the leukotrienes and prostaglandins are not formed until the mast cell undergoes degranulation and the enzymatic breakdown of phospholipids in the plasma membrane. An ensuing enzymatic cascade generates the prostaglandins and the leukotrienes (see Figure 16-6). It therefore takes a longer time for the biological effects of these mediators to become apparent. Their effects are more pronounced and longer lasting, however, than those of histamine. The leukotrienes mediate bronchoconstriction, increased vascular permeability, and mucus production. Prostaglandin D_2 causes bronchoconstriction.

The contraction of human bronchial and tracheal smooth muscles appears at first to be mediated by histamine, but, within 30–60 s, further contraction is mediated by the leukotrienes and prostaglandins. Being active at nanomole levels, the leukotrienes are as much as 1000 times more potent as

TABLE 16-3 Principal mediators involved in type I hypersensitivity

Mediator	Effects
	PRIMARY
Histamine, heparin	Increased vascular permeability; smooth-muscle contraction
Serotonin	Increased vascular permeability; smooth-muscle contraction
Eosinophil chemotactic factor (ECF-A)	Eosinophil chemotaxis
Neutrophil chemotactic factor (NCF-A)	Neutrophil chemotaxis
Proteases	Bronchial mucus secretion; degradation of blood-vessel basement membrane; generation of complement split products
	SECONDARY
Platelet-activating factor	Platelet aggregation and degranulation; contraction of pulmonary smooth muscles
Leukotrienes (slow reactive substance of anaphylaxis, SRS-A)	Increased vascular permeability; contraction of pulmonary smooth muscles
Prostaglandins	Vasodilation; contraction of pulmonary smooth muscles; platelet aggregation
Bradykinin	Increased vascular permeability; smooth-muscle contraction
Cytokines	
IL-1 and TNF-α	Systemic anaphylaxis; increased expression of CAMs on venular endothelial cells
IL-2, IL-3, IL-4, IL-5, IL-6, TGF-β, and GM-CSF	Various effects (see Table 12-1)

bronchoconstrictors than histamine is, and they are also more potent stimulators of vascular permeability and mucus secretion. In humans, the leukotrienes are thought to contribute to the prolonged bronchospasm and buildup of mucus seen in asthmatics.

CYTOKINES

Adding to the complexity of the type I reaction is the variety of cytokines released from mast cells and eosinophils. Some of these may contribute to the clinical manifestations of type I hypersensitivity. Human mast cells secrete IL-4, IL-5, IL-6, and TNF-α These cytokines alter the local microenvironment, eventually leading to the recruitment of inflammatory cells such as neutrophils and eosinophils. IL-4 increases IgE production by B cells. IL-5 is especially important in the recruitment and activation of eosinophils. The high concentrations of TNF-α secreted by mast cells may contribute to shock in systemic anaphylaxis. (This effect may parallel the role of TNF-α in bacterial septic shock and toxic-shock syndrome described in Chapter 12.)

Type I Reactions Can Be Systemic or Localized

The clinical manifestations of type I reactions can range from life-threatening conditions, such as systemic anaphylaxis and asthma, to hay fever and eczema, which are merely annoying.

SYSTEMIC ANAPHYLAXIS

Systemic anaphylaxis is a shock-like and often fatal state whose onset occurs within minutes of a type I hypersensitive reaction. This was the response observed by Portier and Richet in dogs after antigenic challenge. Systemic anaphylaxis can be induced in a variety of experimental animals and is seen occasionally in humans. Each species exhibits characteristic symptoms, which reflect differences in the distribution of mast cells and in the biologically active contents of their granules. The animal model of choice for studying systemic anaphylaxis has been the guinea pig. Anaphylaxis can be induced in guinea pigs with ease, and its symptoms closely parallel those observed in humans.

Active sensitization in guinea pigs is induced by a single injection of a foreign protein such as egg albumin. After an incubation period of about 2 weeks, the animal is usually challenged with an intravenous injection of the same protein. Within 1 min, the animal becomes restless, its respiration becomes labored, and its blood pressure drops. As the smooth muscles of the gastrointestinal tract and bladder contract, the guinea pig defecates and urinates. Finally bronchiole constriction results in death by asphyxiation within 2–4 min of the injection. These events all stem from the systemic vasodilation and smooth-muscle contraction brought on by mediators released in the course of the reaction. Postmortem examination reveals that massive edema, shock, and bronchiole constriction are the major causes of death.

Systemic anaphylaxis in humans is characterized by a similar sequence of events. A wide range of antigens have been shown to trigger this reaction in susceptible humans, including the venom from bee, wasp, hornet, and ant stings; drugs, such as penicillin, insulin, and antitoxins; and seafood and nuts. If not treated quickly, these reactions can be fatal. Epinephrine is the drug of choice for systemic anaphylactic reactions. Epinephrine counteracts the effects of mediators such

as histamine and the leukotrienes by relaxing the smooth muscles and reducing vascular permeability. Epinephrine also improves cardiac output, which is necessary to prevent vascular collapse during an anaphylactic reaction. In addition, epinephrine increases cAMP levels in the mast cell, thereby blocking further degranulation.

LOCALIZED ANAPHYLAXIS (ATOPY)

In localized anaphylaxis, the reaction is limited to a specific target tissue or organ, often involving epithelial surfaces at the site of allergen entry. The tendency to manifest localized anaphylactic reactions is inherited and is called *atopy.* Atopic allergies, which afflict at least 20% of the population in developed countries, include a wide range of IgE-mediated disorders, including allergic rhinitis (hay fever), asthma, atopic dermatitis (eczema), and food allergies.

ALLERGIC RHINITIS The most common atopic disorder, affecting 10% of the U.S. population, is allergic rhinitis, commonly known as hay fever. This results from the reaction of airborne allergens with sensitized mast cells in the conjunctivae and nasal mucosa to induce the release of pharmacologically active mediators from mast cells; these mediators then cause localized vasodilation and increased capillary permeability. The symptoms include watery exudation of the conjunctivae, nasal mucosa, and upper respiratory tract, as well as sneezing and coughing.

ASTHMA Another common manifestation of localized anaphylaxis is asthma. In some cases, airborne or blood-borne allergens, such as pollens, dust, fumes, insect products, or viral antigens, trigger an asthmatic attack (allergic asthma); in other cases, an asthmatic attack can be induced by exercise or cold, apparently independently of allergen stimulation (intrinsic asthma). Like hay fever, asthma is triggered by degranulation of mast cells with release of mediators, but instead of occurring in the nasal mucosa, the reaction develops in the lower respiratory tract. The resulting contraction of the bronchial smooth muscles leads to bronchoconstriction. Airway edema, mucus secretion, and inflammation contribute to the bronchial constriction and to airway obstruction. Asthmatic patients may have abnormal levels of receptors for neuropeptides. For example, asthmatic patients have been reported to have increased expression of receptors for substance P, a peptide that contracts smooth muscles, and decreased expression of receptors for vasoactive intestinal peptide, which relaxes smooth muscles.

Most clinicians view asthma as primarily an inflammatory disease. The asthmatic response can be divided into early and late responses (Figure 16-8). The early response occurs within minutes of allergen exposure and primarily involves histamine, leukotrienes (LTC_4), and prostaglandin (PGD_2). The effects of these mediators lead to bronchoconstriction, vasodilation, and some buildup of mucus. The late response occurs hours later and involves additional mediators, including IL-4, IL-5, IL-16, TNF-α, eosinophil chemotactic factor (ECF), and platelet-activating factor (PAF). The overall effects of these mediators is to increase endothelial cell adhesion as well as to recruit inflammatory cells, including eosinophils and neutrophils, into the bronchial tissue.

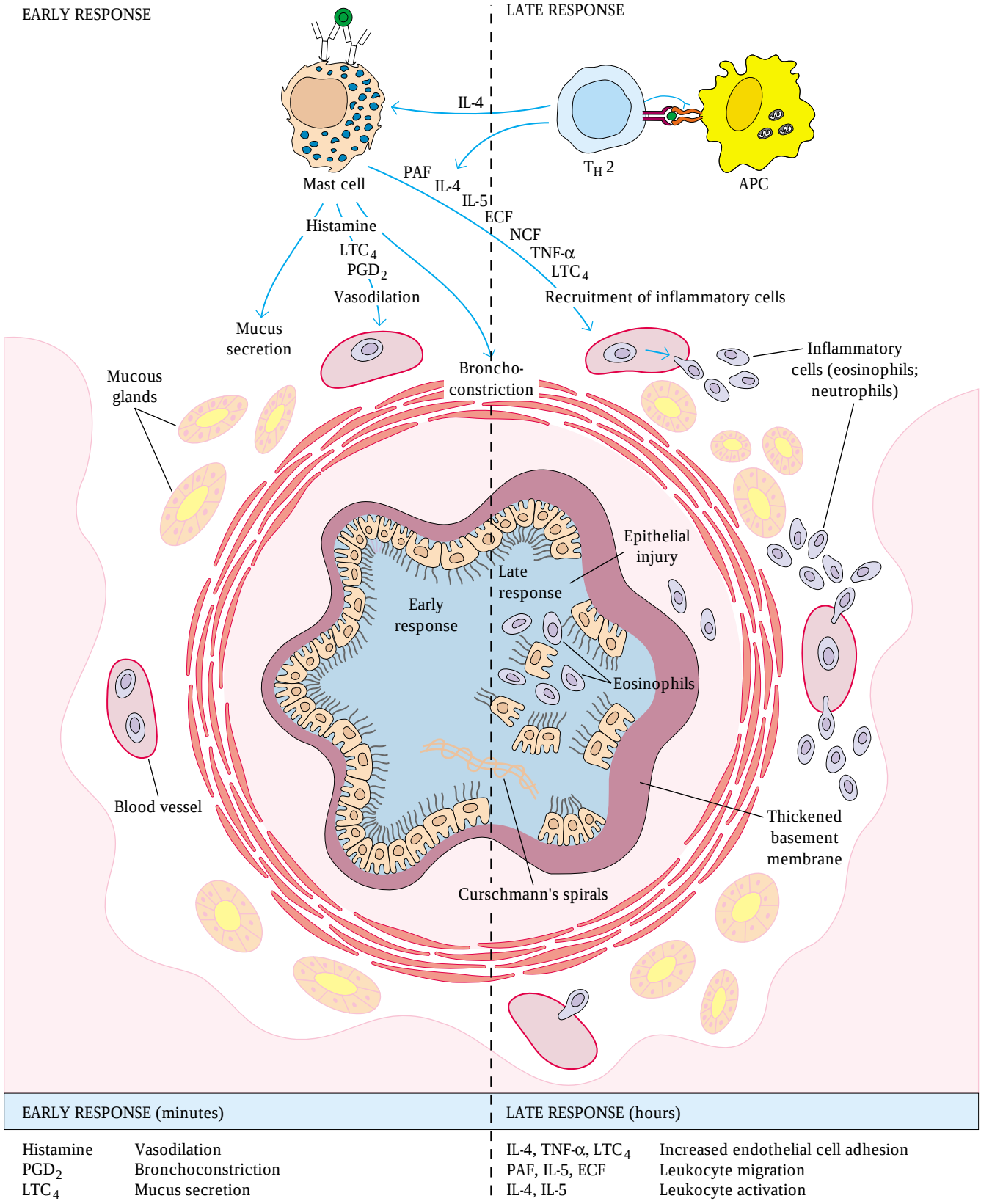
The neutrophils and eosinophils are capable of causing significant tissue injury by releasing toxic enzymes, oxygen radicals, and cytokines. These events lead to occlusion of the bronchial lumen with mucus, proteins, and cellular debris; sloughing of the epithelium; thickening of the basement membrane; fluid buildup (edema); and hypertrophy of the bronchial smooth muscles. A mucus plug often forms and adheres to the bronchial wall. The mucus plug contains clusters of detached epithelial-cell fragments, eosinophils, some neutrophils, and spirals of bronchial tissue known as Curschmann's spirals. Asthma is increasing in prevalence in the United States, particularly among children in inner-city environments (see Clinical Focus on page 376).

FOOD ALLERGIES Various foods also can induce localized anaphylaxis in allergic individuals. Allergen crosslinking of IgE on mast cells along the upper or lower gastrointestinal tract can induce localized smooth-muscle contraction and vasodilation and thus such symptoms as vomiting or diarrhea. Mast-cell degranulation along the gut can increase the permeability of mucous membranes, so that the allergen enters the bloodstream. Various symptoms can ensue, depending on where the allergen is deposited. For example, some individuals develop asthmatic attacks after ingesting certain foods. Others develop atopic urticaria, commonly known as hives, when a food allergen is carried to sensitized mast cells in the skin, causing swollen (edematous) red (erythematous) eruptions; this is the wheal and flare response, or P-K reaction, mentioned earlier.

ATOPIC DERMATITIS Atopic dermatitis (allergic eczema) is an inflammatory disease of skin that is frequently associated with a family history of atopy. The disease is observed most frequently in young children, often developing during infancy. Serum IgE levels are often elevated. The allergic individual develops skin eruptions that are erythematous and filled with pus. Unlike a delayed-type hypersensitive reaction, which involves T_H1 cells, the skin lesions in atopic dermatitis have T_H2 cells and an increased number of eosinophils.

Late-Phase Reactions Induce Localized Inflammatory Reactions

As a type I hypersensitive reaction begins to subside, mediators released during the course of the reaction often induce localized inflammation called the late-phase reaction. Distinct from the late response seen in asthma, the late-phase reaction begins to develop 4–6 h after the initial type I reaction and persists for 1–2 days. The reaction is characterized by infiltration of neutrophils, eosinophils, macrophages, lymphocytes, and basophils. The localized late-phase response also may be mediated partly by cytokines released from mast cells.



EARLY RESPONSE (minutes)		LATE RESPONSE (hours)	
Histamine	Vasodilation	IL-4, TNF-α, LTC_4	Increased endothelial cell adhesion
PGD_2	Bronchoconstriction	PAF, IL-5, ECF	Leukocyte migration
LTC_4	Mucus secretion	IL-4, IL-5	Leukocyte activation

FIGURE 16-8 The early and late inflammatory responses in asthma. The immune cells involved in the early and late responses are represented at the top. The effects of various mediators on an airway, represented in cross section, are illustrated in the center.

Both TNF-α and IL-1 increase the expression of cell-adhesion molecules on venular endothelial cells, thus facilitating the buildup of neutrophils, eosinophils, and monocytes that characterizes the late-phase response.

Eosinophils play a principal role in the late-phase reaction, accounting for some 30% of the cells that accumulate. Eosinophil chemotactic factor, released by mast cells during the initial reaction, attracts large numbers of eosinophils to the affected site. Various cytokines released at the site, including IL-3, IL-5, and GM-CSF, contribute to the growth and differentiation of the eosinophils. Eosinophils express Fc receptors for IgG and IgE isotypes and bind directly to antibody-coated allergen. Much as in mast-cell degranulation, binding of antibody-coated antigen activates eosinophils, leading to their degranulation and release of inflammatory mediators, including leukotrienes, major basic protein, platelet-activation factor, eosinophil cationic protein (ECP), and eosinophil-derived neurotoxin. The release of these eosinophil-derived mediators may play a protective role in parasitic infections. However, in response to allergens, these mediators contribute to extensive tissue damage in the late-phase reaction. The influx of eosinophils in the late-phase response has been shown to contribute to the chronic inflammation of the bronchial mucosa that characterizes persistent asthma.

Neutrophils are another major participant in late-phase reactions, accounting for another 30% of the inflammatory cells. Neutrophils are attracted to the area of a type I reaction by neutrophil chemotactic factor, released from degranulating mast cells. In addition, a variety of cytokines released at the site, including IL-8, have been shown to activate neutrophils, resulting in release of their granule contents, including lytic enzymes, platelet-activating factor, and leukotrienes.

Type I Responses Are Regulated by Many Factors

As noted earlier, the antigen dose, mode of antigen presentation, and genetic constitution of an animal influence the level of the IgE response induced by an antigen (i.e., its allergenicity). Breeding experiments with mice have shown that this genetic variation is not linked to the MHC. A genetic component also has been shown to influence susceptibility to type I hypersensitive reactions in humans. If both parents are allergic, there is a 50% chance that a child will also be allergic; when only one parent is allergic, there is a 30% chance that a child will manifest some kind of type I reaction.

The effect of antigen dosage on the IgE response is illustrated by immunization of BDF1 mice. Repeated low doses of an appropriate antigen induce a persistent IgE response in these mice, but higher antigen doses result in transient IgE production and a shift toward IgG. The mode of antigen presentation also influences the development of the IgE response. For example, immunization of Lewis-strain rats with keyhole limpet hemocyanin (KLH) plus aluminum hydroxide gel or *Bordetella pertussis* as an adjuvant induces a strong IgE response, whereas injection of KLH with complete Freund's adjuvant produces a largely IgG response. Infection of mice with the nematode *Nippostrongylus brasiliensis* (Nb), like certain adjuvants, preferentially induces an IgE response. For example, Nb-infected mice develop higher levels of IgE specific for an unrelated antigen than do uninfected control mice.

The relative levels of the T_H1 and T_H2 subsets also are key to the regulation of type I hypersensitive responses. T_H1 cells reduce the response, whereas T_H2 cells enhance it. Cytokines secreted by T_H2 cells—namely, IL-3, IL-4, IL-5, and IL-10—stimulate the type I response in several ways. IL-4 enhances class switching to IgE and regulates the clonal expansion of IgE-committed B cells; IL-3, IL-4, and IL-10 enhance mast-cell production; and IL-3 and IL-5 enhance eosinophil maturation, activation, and accumulation. In contrast, T_H1 cells produce IFN-γ which inhibits the type I response.

The pivotal role of IL-4 in regulation of the type I response was demonstrated in experiments by W. E. Paul and coworkers. When these researchers activated normal, unprimed B cells in vitro with the bacterial endotoxin lipopolysaccharide (LPS), only 2% of the cells expressed membrane IgG1 and only 0.05% expressed membrane IgE. However, when unprimed B cells were incubated with LPS plus IL-4, the percentage of cells expressing IgG1 increased to 40%–50% and the percentage expressing IgE increased to 15%–25%. In an attempt to determine whether IL-4 plays a role in regulating IgE production in vivo, Paul primed Nb-infected mice with the harmless antigen TNP-KLH in the presence and absence of monoclonal antibody to IL-4. The antibody to IL-4 reduced the production of IgE specific for TNP-KLH in these Nb-infected mice to 1% of the level in control animals.

Further support for the role of IL-4 in the IgE response comes from the experiments of K. Rajewsky and coworkers with IL-4 knockout mice. These IL-4–deficient mice were unable to mount an IgE response to helminthic antigens. Furthermore, increased levels of $CD4^+$ T_H2 cells and increased levels of IL-4 have been detected in atopic individuals. When allergen-specific $CD4^+$ T cells from atopic individuals are cloned and added to an autologous B-cell culture, the B cells synthesize IgE, whereas allergen-specific $CD4^+$ T cells from nonatopic individuals do not support IgE production.

In contrast to IL-4, IFN-γ decreases IgE production, suggesting that the balance of IL-4 and IFN-γ may determine the amount of IgE produced (Figure 16-9). Since IFN-γ is secreted by the T_H1 subset and IL-4 by the T_H2 subset, the relative activity of these subsets may influence an individual's response to allergens. According to this proposal, atopic and nonatopic individuals would exhibit qualitatively different type I responses to an allergen: the response in atopic individuals would involve the T_H2 subset and result in production of IgE; the response in nonatopic individuals would involve the T_H1 subset and result in production of IgM or IgG. To test this hypothesis, allergen-specific T cells were cloned from atopic and nonatopic individuals. The cloned T cells from the

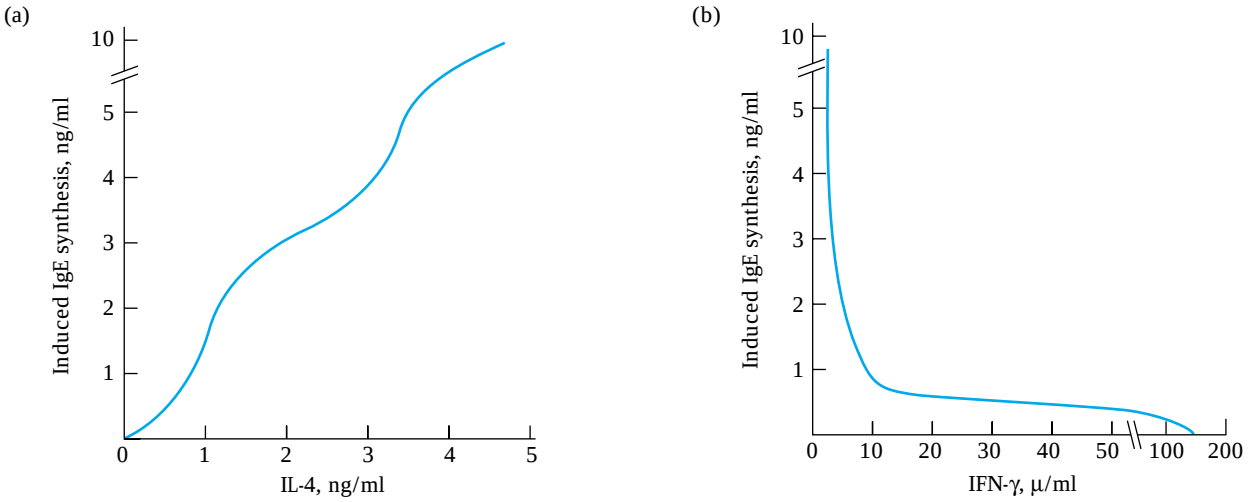


FIGURE 16-9 Effect of IL-4 and IFN-γ on in vitro production of IgE. These plots show the amount of IgE produced by plasma cells cultured in the presence of various concentrations of IL-4 (a) or IFN-γ (b). *[Adapted from G. Del Prete, 1988,* J. Immunol. ***140:****4193.]*

atopic individuals were predominantly of the T_H2 phenotype (secreting IL-4), whereas the cloned T cells from nonatopic individuals were predominantly of the T_H1 phenotype (secreting IFN-γ). Needless to say, there is keen interest in down-regulating IL-4 as a possible treatment for allergic individuals.

Several Methods Are Used to Detect Type I Hypersensitivity Reactions

Type I hypersensitivity is commonly identified and assessed by skin testing. Small amounts of potential allergens are introduced at specific skin sites either by intradermal injection or by superficial scratching. A number of tests can be applied to sites on the forearm or back of an individual at one time. If a person is allergic to the allergen, local mast cells degranulate and the release of histamine and other mediators produces a wheal and flare within 30 min (Figure 16-10). The advantage of skin testing is that it is relatively inexpensive and allows screening of a large number of allergens at one time. The disadvantage of skin testing is that it sometimes sensitizes the allergic individual to new allergens and in some rare cases may induce systemic anaphylactic shock. A few individuals also manifest a late-phase reaction, which comes 4–6 h after testing and sometimes lasts for up to 24 h. As noted already, eosinophils accumulate during a late-phase reaction, and release of eosinophil-granule contents contributes to the tissue damage in a late-phase reaction site.

Another method of assessing type I hypersensitivity is to determine the serum level of total IgE antibody by the radioimmunosorbent test (RIST). This highly sensitive technique, based on the radioimmunoassay, can detect nanomolar levels of total IgE. The patient's serum is reacted with agarose beads or paper disks coated with rabbit anti-IgE. After the beads or disks are washed, ^{125}I-labeled rabbit anti-IgE is added. The radioactivity of the beads or disks, measured with a gamma counter, is proportional to the level of IgE in the patient's serum (Figure 16-11a).

The similar radioallergosorbent test (RAST) detects the serum level of IgE specific for a given allergen. The allergen is coupled to beads or disks, the patient's serum is added, and

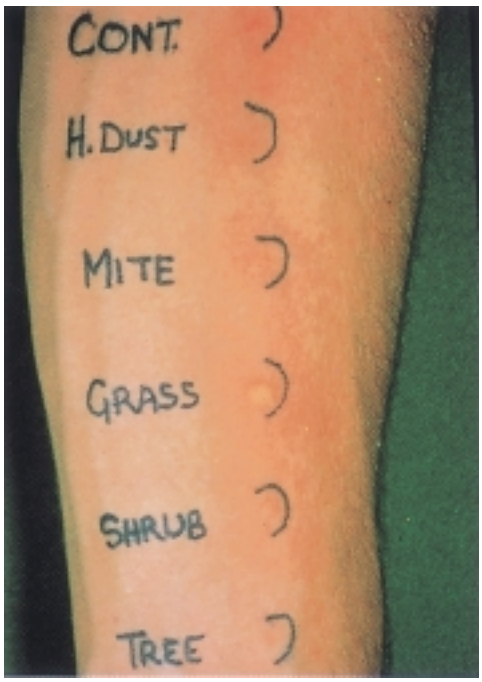


FIGURE 16-10 Skin testing by intradermal injection of allergens into the forearm. In this individual, a weal and flare response developed within a few minutes at the site where grass was injected, indicating that the individual is allergic to grass. *[From L. M. Lichtenstein, 1993,* Sci. Am. ***269****(2):117. Used with permission.]*

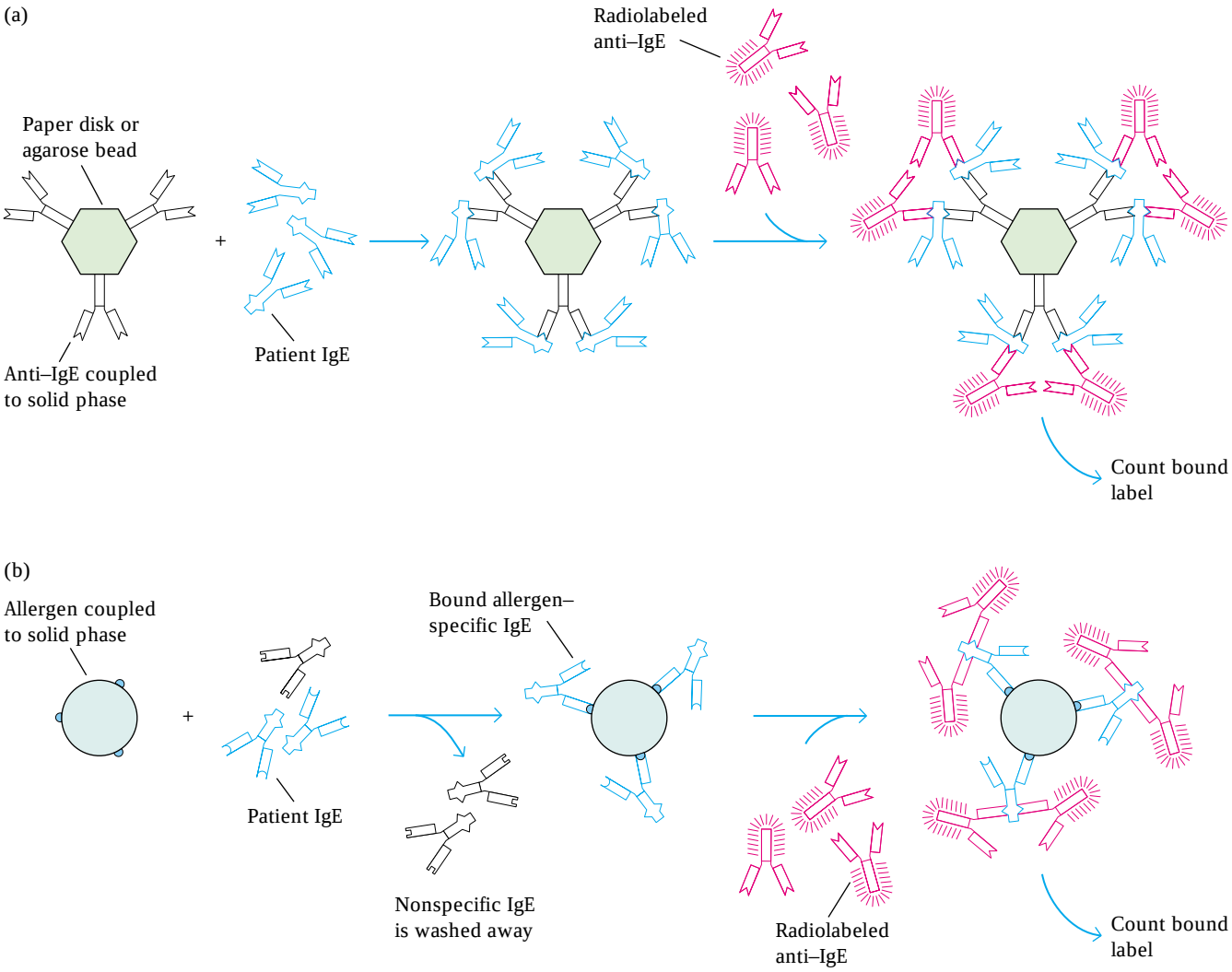


FIGURE 16-11 Procedures for assessing type I hypersensitivity. (a) Radioimmunosorbent test (RIST) can quantify nanogram amounts of total serum IgE. (b) Radioallergosorbent test (RAST) can quantify nanogram amounts of serum IgE specific for a particular allergen.

unbound antibody is washed away. The amount of specific IgE bound to the solid-phase allergen is then measured by adding ^{125}I-labeled rabbit anti-IgE, washing the beads, and counting the bound radioactivity (Figure 16-11b).

Type I Hypersensitivities Can Be Controlled Medically

The obvious first step in controlling type I hypersensitivities is to avoid contact with known allergens. Often the removal of house pets, dust-control measures, or avoidance of offending foods can eliminate a type I response. Elimination of inhalant allergens (such as pollens) is a physical impossibility, however, and other means of intervention must be pursued.

Immunotherapy with repeated injections of increasing doses of allergens (hyposensitization) has been known for some time to reduce the severity of type I reactions, or even eliminate them completely, in a significant number of individuals suffering from allergic rhinitis. Such repeated introduction of allergen by subcutaneous injections appears to cause a shift toward IgG production or to induce T-cell–mediated suppression (possibly by a shift to the T_H1 subset and IFN-γ production) that turns off the IgE response (Figure 16-12). In this situation, the IgG antibody is referred to as *blocking antibody* because it competes for the allergen, binds to it, and forms a complex that can be removed by phagocytosis; as a result, the allergen is not available to crosslink the fixed IgE on the mast-cell membranes, and allergic symptoms decrease.

Another form of immunotherapy is the use of humanized monoclonal anti-IgE. These antibodies bind to IgE, but only if IgE is not already bound to FcεRI; the latter would lead to histamine release. In fact, the monoclonal antibodies are specifically selected to bind membrane IgE on IgE-expressing B cells.

CLINICAL FOCUS

The Genetics of Asthma

Asthma affects almost 5% of the population of the United States. For reasons that are still unclear, the incidence of asthma recently has increased dramatically in developed countries. Even more alarming is that the severity of the disease also appears to be increasing. The increase in asthma mortality is highest among children, and in the United States the mortality is highest among African-American children of the inner city. In 1999, 7.7 million children had asthma and more than 2000 of them died of the disease. These statistics are increasing each year. In addition to its human costs, asthma imposes high financial costs on society. During 2000, the cost for the treatment of asthma in the United States was more than $12 billion.

Asthma is commonly defined as an inflammatory disease of the airway, and it is characterized by bronchial hyperresponsiveness. Atopic individuals, those with a predisposition to the type I hypersensitive response, are most susceptible to the development of bronchial hyperresponsiveness and asthma, but only 10%–30% of atopic individuals actually develop asthma. The evidence that asthma has a genetic component originally was derived from family studies, which estimated that the relative contribution of genetic factors to atopy and asthma is 40%–60%. While genetic factors are important, further studies have indicated that environmental factors also play a large role. Additionally, asthma is a complex genetic disease, controlled by several genes, so that susceptibility to it is likely to involve the interaction of multiple genetic and environmental factors.

How do we determine which genes contribute to a complex multigenic disease such as this? One approach is the candidate-gene approach, in which a hypothesis suggests that a particular gene or set of genes may have some relation to the disease. After such a gene has been identified, families with apparent predisposition to the disease are examined for polymorphic alleles of the gene in question. Comparing family members who do or do not have the disease allows correlation between a particular allele and the presence of the disease. The problem with this approach is its bias toward identification of genes already suspected to play a role in the disease, which precludes identification of new genes. A good example of the use of the candidate-gene approach is the identification of a region on chromosome 5, region 5q31–33, that appears to be linked to the development of asthma. Using a candidate-gene approach, this region was investigated because it includes a cluster of cytokine genes, among them the genes that encode IL-3, -4, -5, -9, and -13, as well as the gene that encodes granulocytemacrophage colony-stimulating factor. *IL-4* is thought to be a good candidate gene, since it induces the Ig class-switch to IgE. Several groups of investigators have examined this region in different populations and concluded that there is a polymorphism associ-

These antibodies are humanized by the genetic engineering of the genes encoding the H and L chains; mouse framework regions are replaced with human framework sequences and the end result is a mouse/human chimeric monoclonal that is not likely to be recognized as foreign by the human immune system. When injected into people suffering from allergy, these antibodies can bind free IgE as well as down-regulate IgE production in B cells. This results in lower serum IgE concentration which, in turn, reduces the sensitivity of basophils. This form of immunotherapy is useful in treating many forms of allergies, especially crippling food allergies.

Another approach for treating allergies stems from the finding that soluble antigens tend to induce a state of anergy by activating T cells in the absence of the necessary costimulatory signal (see Figure 10-15). Presumably, a soluble

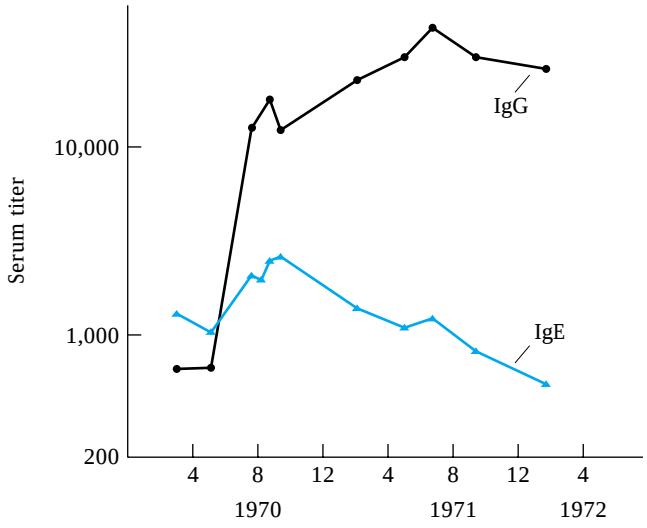


FIGURE 16-12 Hyposensitization treatment of type I allergy. Injection of ragweed antigen periodically for 2 years into a ragweed-sensitive individual induced a gradual decrease in IgE levels and a dramatic increase in IgG. Both antibodies were measured by a radioimmunoassay. *[Adapted from K. Ishizaka and T. Ishizaka, 1973, in* Asthma Physiology, Immunopharmacology and Treatment, *K. F. Austen and L. M. Lichtenstein (eds.), Academic Press.]*

ated with predisposition to asthma that maps to the promotor region of *IL-4.* Additionally, two alleles of *IL-9* associated with atopy have been identified.

Another approach to identifying genes associated with a particular disease is a random genomic search. In this method, the entire genome is scanned for polymorphisms associated with the disease in question. Using the random genomic approach, a British study (Lympany et al., 1992) identified a linkage between a polymorphism on chromosome 11—more specifically, region 11q13—associated with atopy in British families. This region maps to the vicinity of the β subunit of the high-affinity IgE receptor (FcεRIβ). This association is exciting, since we know how important IgE is in mediating type I reactions. However, some caution in interpreting these results is necessary. This study looked at associations between polymorphisms and atopy, but most individuals who are atopic do not develop asthma. Therefore this association, while important in identifying factors in developing atopy, may not be relevant to the development of asthma.

More recently, a large genome-wide screen for loci linked to asthma susceptibility was conducted in ethnically diverse populations that included Caucasians, Hispanics, and African-Americans. This study, published by a large collaborative group from medical centers throughout the United States identified many candidate loci associated with asthma. One locus on chromosome 5 coincided with the already identified region at 5q31–33. Interestingly, however, this locus was associated with asthma in Caucasians but not in Hispanics or African-Americans. Similarly, some loci appeared to have a high correlation with asthma in Hispanics only, and other loci were identified as unique to African-Americans. Another interesting conclusion was that the association between chromosome 11q and atopy did not appear to be correlated with asthma. This could indicate that asthma and atopy have different molecular bases. More important, it suggests that genetic linkage to atopy should not be confused with genetic linkage to asthma. Overall, this study identified several genes linked to asthma and found that the number and relative importance of these genes may differ among ethnic groups. This suggests that genetic differences as well as differences in environment may be the underlying basis of the differences observed in the prevalence as well as the severity of the disease among ethnic groups in the United States.

It is well documented that a higher than average percentage of African-American inner-city children have serious complications with asthma. This has raised the question whether there is a genetic predisposition for asthma in African-Americans. Recently, however, a report from Rosenstreich and colleagues has indicated an important environmental linkage to asthma in the inner city. This group assessed the role of allergies to the cockroach in the development of asthma; they found that a combination of cockroach allergy and exposure to high levels of cockroach allergen can help explain the high frequency of asthma-related health problems in inner-city children. These data also point to defects in the public-health systems in large cities. Clearly, a concerted effort by public agencies to eradicate insect infestations would benefit the health of those who live in inner-city communities.

antigen is internalized by endocytosis, processed, and presented with class II MHC molecules, but fails to induce expression of the requisite co-stimulatory ligand (B7) on antigen-presenting cells.

Knowledge of the mechanism of mast-cell degranulation and the mediators involved in type I reactions opened the way to drug therapy for allergies. Antihistamines have been the most useful drugs for symptoms of allergic rhinitis. These drugs act by binding to the histamine receptors on target cells and blocking the binding of histamine. The H_1 receptors are blocked by the classical antihistamines, and the H_2 receptors by a newer class of antihistamines.

Several drugs block release of allergic mediators by interfering with various biochemical steps in mast-cell activation and degranulation (Table 16-4). Disodium cromoglycate (cromolyn sodium) prevents Ca^{2+} influx into mast cells. Theophylline, which is commonly administered to asthmatics orally or through inhalers, blocks phosphodiesterase, which catalyzes the breakdown of cAMP to 5′-AMP. The resulting prolonged increase in cAMP levels blocks degranulation. A number of drugs stimulate the β-adrenergic system by stimulating β-adrenergic receptors. As mentioned earlier,

TABLE 16-4 Mechanism of action of some drugs used to treat type I hypersensitivity

Drug	Action
Antihistamines	Block H_1 and H_2 receptors on target cells
Cromolyn sodium	Blocks Ca^{2+} influx into mast cells
Theophylline	Prolongs high cAMP levels in mast cells by inhibiting phosphodiesterase, which cleaves cAMP to 5′-AMP*
Epinephrine (adrenalin)	Stimulates cAMP production by binding to β-adrenergic receptors on mast cells*
Cortisone	Reduces histamine levels by blocking conversion of histidine to histamine and stimulates mast-cell production of cAMP*

*Although cAMP rises transiently during mast-cell activation, degranulation is prevented if cAMP levels remain high.

epinephrine (also known as adrenaline) is commonly administered during anaphylactic shock. It acts by binding to β-adrenergic receptors on bronchial smooth muscles and mast cells, elevating the cAMP levels within these cells. The increased levels of cAMP promote relaxation of the bronchial muscles and decreased mast-cell degranulation. A number of epinephrine analogs have been developed that bind to selected β-adrenergic receptors and induce cAMP increases with fewer side effects than are seen with epinephrine. Cortisone and various other anti-inflammatory drugs also have been used to reduce type I reactions.

Antibody-Mediated Cytotoxic (Type II) Hypersensitivity

Type II hypersensitive reactions involve antibody-mediated destruction of cells. Antibody can activate the complement system, creating pores in the membrane of a foreign cell (see Figure 13-5), or it can mediate cell destruction by antibody-dependent cell-mediated cytotoxicity (ADCC). In this process, cytotoxic cells with Fc receptors bind to the Fc region of antibodies on target cells and promote killing of the cells (see Figure 14-12). Antibody bound to a foreign cell also can serve as an opsonin, enabling phagocytic cells with Fc or C3b receptors to bind and phagocytose the antibody-coated cell (see Figure 13-12).

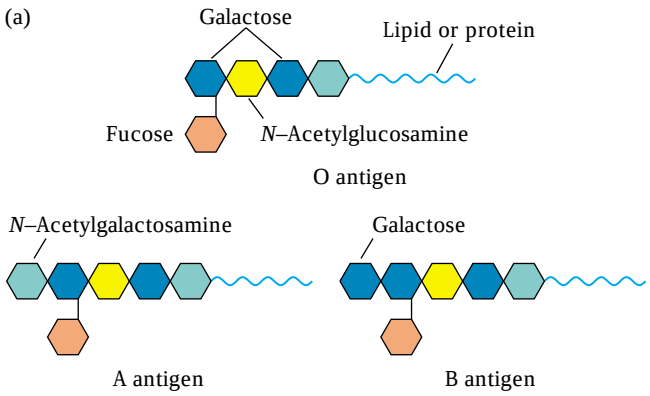
This section examines three examples of type II hypersensitive reactions. Certain autoimmune diseases involve autoantibody–mediated cellular destruction by type II mechanisms. These diseases are described in Chapter 20.

Transfusion Reactions Are Type II Reactions

A large number of proteins and glycoproteins on the membrane of red blood cells are encoded by different genes, each of which has a number of alternative alleles. An individual possessing one allelic form of a blood-group antigen can recognize other allelic forms on transfused blood as foreign and mount an antibody response. In some cases, the antibodies have already been induced by natural exposure to similar antigenic determinants on a variety of microorganisms present in the normal flora of the gut. This is the case with the ABO blood-group antigens (Figure 16-13a).

Antibodies to the A, B, and O antigens, called isohemagglutinins, are usually of the IgM class. An individual with blood type A, for example, recognizes B-like epitopes on intestinal microorganisms and produces isohemagglutinins to the B-like epitopes. This same individual does not respond to A-like epitopes on the same intestinal microorganisms because these A-like epitopes are too similar to self and a state of self-tolerance to these epitopes should exist (Figure 16-13b). If a type A individual is transfused with blood containing type B cells, a **transfusion reaction** occurs in which the anti-B isohemagglutinins bind to the B blood cells and mediate their

(a)



(b)

Genotype	Blood–group phenotype	Antigens on erythrocytes (*agglutinins*)	Serum antibodies (*isohemagglutinins*)
AA or AO	A	A	Anti–B
BB or BO	B	B	Anti–A
AB	AB	A and B	None
OO	O	None	Anti–A and anti–B

FIGURE 16-13 ABO blood group. (a) Structure of terminal sugars, which constitute the distinguishing epitopes, in the A, B, and O blood antigens. (b) ABO genotypes and corresponding phenotypes, agglutinins, and isohemagglutinins.

destruction by means of complement-mediated lysis. Antibodies to other blood-group antigens may result from repeated blood transfusions because minor allelic differences in these antigens can stimulate antibody production. These antibodies are usually of the IgG class.

The clinical manifestations of transfusion reactions result from massive intravascular hemolysis of the transfused red blood cells by antibody plus complement. These manifestations may be either immediate or delayed. Reactions that begin immediately are most commonly associated with ABO blood-group incompatibilities, which lead to complement-mediated lysis triggered by the IgM isohemagglutinins. Within hours, free hemoglobin can be detected in the plasma; it is filtered through the kidneys, resulting in hemoglobinuria. Some of the hemoglobin gets converted to bilirubin, which at high levels is toxic. Typical symptoms include fever, chills, nausea, clotting within blood vessels, pain in the lower back, and hemoglobin in the urine. Treatment involves prompt termination of the transfusion and maintenance of urine flow with a diuretic, because the accumulation of hemoglobin in the kidney can cause acute tubular necrosis.

Delayed hemolytic transfusion reactions generally occur in individuals who have received repeated transfusions of ABO-compatible blood that is incompatible for other blood-group antigens. The reactions develop between 2 and 6 days after transfusion, reflecting the secondary nature of these reactions. The transfused blood induces clonal selection and production of IgG against a variety of blood-group membrane antigens, most commonly Rh, Kidd, Kell, and Duffy. The predominant isotype involved in these reactions is IgG, which is less effective than IgM in activating complement. For this reason, complement-mediated lysis of the transfused red blood cells is incomplete, and many of the transfused cells are destroyed at extravascular sites by agglutination, opsonization, and subsequent phagocytosis by macrophages. Symptoms include fever, low hemoglobin, increased bilirubin, mild jaundice, and anemia. Free hemoglobin is usually not detected in the plasma or urine in these reactions because RBC destruction occurs in extravascular sites.

Hemolytic Disease of the Newborn Is Caused by Type II Reactions

Hemolytic disease of the newborn develops when maternal IgG antibodies specific for fetal blood-group antigens cross the placenta and destroy fetal red blood cells. The consequences of such transfer can be minor, serious, or lethal. Severe hemolytic disease of the newborn, called **erythroblastosis fetalis,** most commonly develops when an Rh^+ fetus expresses an **Rh antigen** on its blood cells that the Rh^- mother does not express.

During pregnancy, fetal red blood cells are separated from the mother's circulation by a layer of cells in the placenta called the trophoblast. During her first pregnancy with an Rh^+ fetus, an Rh^- woman is usually not exposed to enough fetal red blood cells to activate her Rh-specific B cells. At the time of delivery, however, separation of the placenta from the uterine wall allows larger amounts of fetal umbilical-cord blood to enter the mother's circulation. These fetal red blood cells activate Rh-specific B cells, resulting in production of Rh-specific plasma cells and memory B cells in the mother. The secreted IgM antibody clears the Rh^+ fetal red cells from the mother's circulation, but the memory cells remain, a threat to any subsequent pregnancy with an Rh^+ fetus. Activation of these memory cells in a subsequent pregnancy results in the formation of IgG anti-Rh antibodies, which cross the placenta and damage the fetal red blood cells (Figure 16-14). Mild to severe anemia can develop in the fetus, sometimes with fatal consequences. In addition, conversion of hemoglobin to bilirubin can present an additional threat to the newborn because the lipid-soluble bilirubin may accumulate in the brain and cause brain damage.

Hemolytic disease of the newborn caused by Rh incompatibility in a subsequent pregnancy can be almost entirely prevented by administering antibodies against the Rh antigen to the mother within 24–48 h after the first delivery. These antibodies, called **Rhogam,** bind to any fetal red blood cells that enter the mother's circulation at the time of delivery and facilitate their clearance before B-cell activation and ensuing memory-cell production can take place. In a subsequent pregnancy with an Rh^+ fetus, a mother who has been treated with Rhogam is unlikely to produce IgG anti-Rh antibodies; thus, the fetus is protected from the damage that would occur when these antibodies crossed the placenta.

The development of hemolytic disease of the newborn caused by Rh incompatibility can be detected by testing maternal serum at intervals during pregnancy for antibodies to the Rh antigen. A rise in the titer of these antibodies as pregnancy progresses indicates that the mother has been exposed to Rh antigens and is producing increasing amounts of antibody. The presence of maternal IgG on the surface of fetal red blood cells can be detected by a Coombs test. Isolated fetal red blood cells are incubated with the Coombs reagent, goat antibody to human IgG antibody. If maternal IgG is bound to the fetal red blood cells, the cells agglutinate with the Coombs reagent.

If hemolytic disease caused by Rh incompatibility is detected during pregnancy, the treatment depends on the severity of the reaction. For a severe reaction, the fetus can be given an intrauterine blood-exchange transfusion to replace fetal Rh^+ red blood cells with Rh^- cells. These transfusions are given every 10–21 days until delivery. In less severe cases, a blood-exchange transfusion is not given until after birth, primarily to remove bilirubin; the infant is also exposed to low levels of UV light to break down the bilirubin and prevent cerebral damage. The mother can also be treated during the pregnancy by **plasmapheresis.** In this procedure, a cell-separation machine is used to separate the mother's blood into two fractions, cells and plasma. The plasma containing the anti-Rh antibody is discarded, and the cells are reinfused into the mother in an albumin or fresh-plasma solution.

The majority of cases (65%) of hemolytic disease of the newborn have minor consequences and are caused by ABO

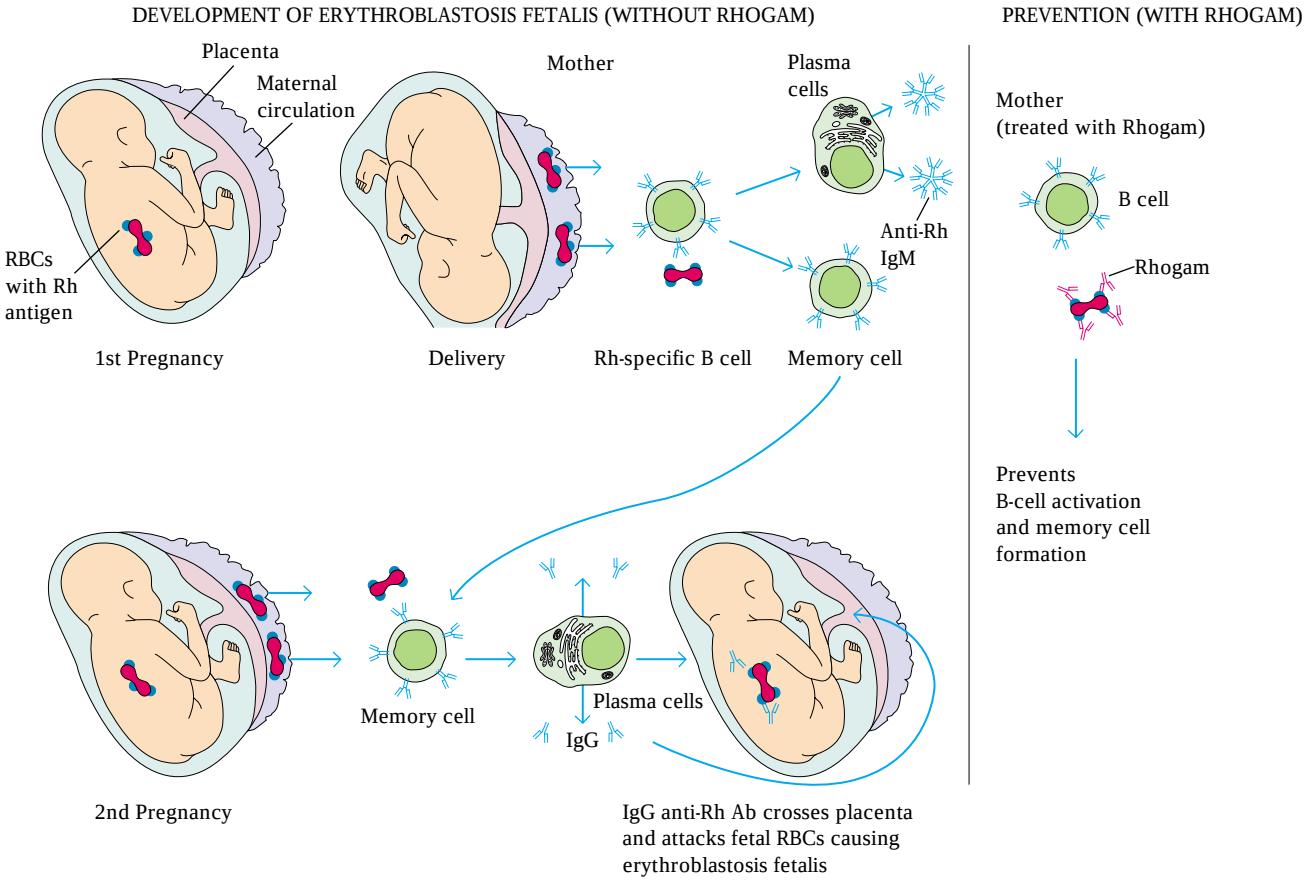


FIGURE 16-14 Development of erythroblastosis fetalis (hemolytic disease of the newborn) caused when an Rh^- mother carries an Rh^+ fetus (*left*), and effect of treatment with anti-Rh antibody, or Rhogam (*right*).

blood-group incompatibility between the mother and fetus. Type A or B fetuses carried by type O mothers most commonly develop these reactions. A type O mother is most likely to develop IgG antibody to the A or B blood-group antigens either through natural exposure or through exposure to fetal blood-group A or B antigens in successive pregnancies. Usually the fetal anemia resulting from this incompatibility is mild; the major clinical manifestation is a slight elevation of bilirubin, with jaundice. Depending on the severity of the anemia and jaundice, a blood-exchange transfusion may be required in these infants. In general the reaction is mild, however, and exposure of the infant to low levels of UV light is enough to break down the bilirubin and avoid cerebral damage.

Drug-Induced Hemolytic Anemia Is a Type II Response

Certain antibiotics (e.g., penicillin, cephalosporin, and streptomycin) can adsorb nonspecifically to proteins on RBC membranes, forming a complex similar to a hapten-carrier complex. In some patients, such drug-protein complexes induce formation of antibodies, which then bind to the adsorbed drug on red blood cells, inducing complement-mediated lysis and thus progressive anemia. When the drug is withdrawn, the hemolytic anemia disappears. Penicillin is notable in that it can induce all four types of hypersensitivity with various clinical manifestations (Table 16-5).

TABLE 16-5 Penicillin-induced hypersensitive reactions

Type of reaction	Antibody or lymphocytes induced	Clinical manifestations
I	IgE	Urticaria, systemic anaphylaxis
II	IgM, IgG	Hemolytic anemia
III	IgG	Serum sickness, glomerulonephritis
IV	T_{DTH} cells	Contact dermatitis

Immune Complex–Mediated (Type III) Hypersensitivity

The reaction of antibody with antigen generates immune complexes. Generally this complexing of antigen with antibody facilitates the clearance of antigen by phagocytic cells. In some cases, however, large amounts of immune complexes can lead to tissue-damaging type III hypersensitive reactions. The magnitude of the reaction depends on the quantity of immune complexes as well as their distribution within the body. When the complexes are deposited in tissue very near the site of antigen entry, a localized reaction develops. When the complexes are formed in the blood, a reaction can develop wherever the complexes are deposited. In particular, complex deposition is frequently observed on blood-vessel walls, in the synovial membrane of joints, on the glomerular basement membrane of the kidney, and on the choroid plexus of the brain. The deposition of these complexes initiates a reaction that results in the recruitment of neutrophils to the site. The tissue there is injured as a consequence of granular release from the neutrophil.

Type III hypersensitive reactions develop when immune complexes activate the complement system's array of immune effector molecules (see Figure 13-2). As explained in Chapter 13, the C3a, C4a, and C5a complement split products are anaphylatoxins that cause localized mast-cell degranulation and consequent increase in local vascular permeability. C3a, C5a, and C5b67 are also chemotactic factors for neutrophils, which can accumulate in large numbers at the site of immune-complex deposition. Larger immune complexes are deposited on the basement membrane of blood-vessel walls or kidney glomeruli, whereas smaller complexes may pass through the basement membrane and be deposited in the subepithelium. The type of lesion that results depends on the site of deposition of the complexes.

Much of the tissue damage in type III reactions stems from release of lytic enzymes by neutrophils as they attempt to phagocytose immune complexes. The C3b complement component acts as an opsonin, coating immune complexes. A neutrophil binds to a C3b-coated immune complex by means of the type I complement receptor, which is specific for C3b. Because the complex is deposited on the basement-membrane surface, phagocytosis is impeded, so that lytic enzymes are released during the unsuccessful attempts of the neutrophil to ingest the adhering immune complex. Further activation of the membrane-attack mechanism of the complement system can also contribute to the destruction of tissue. In addition, the activation of complement can induce aggregation of platelets, and the resulting release of clotting factors can lead to formation of microthrombi.

Type III Reactions Can Be Localized

Injection of an antigen intradermally or subcutaneously into an animal that has high levels of circulating antibody specific for that antigen leads to formation of localized immune complexes, which mediate an acute Arthus reaction within 4–8 h (Figure 16-15). Microscopic examination of the tissue reveals neutrophils adhering to the vascular endothelium and then migrating into the tissues at the site of immune-complex deposition. As the reaction develops, localized tissue and vascular damage results in an accumulation of fluid (edema) and red blood cells (erythema) at the site. The severity of the reaction can vary from mild swelling and redness to tissue necrosis.

After an insect bite, a sensitive individual may have a rapid, localized type I reaction at the site. Often, some 4–8 h later, a typical Arthus reaction also develops at the site, with pronounced erythema and edema. Intrapulmonary Arthus-type

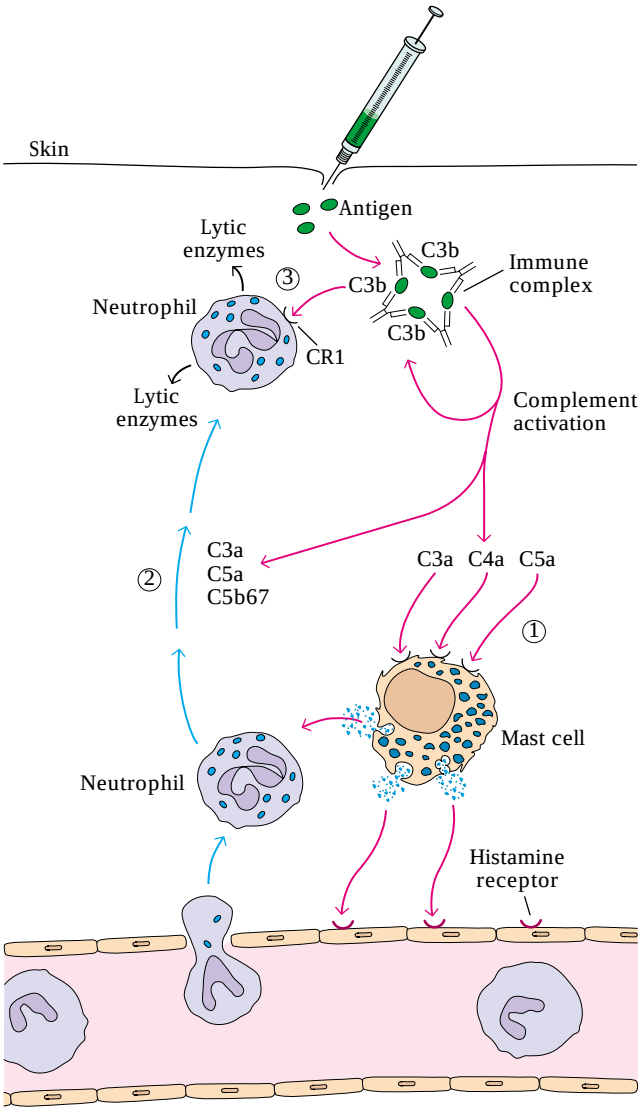


FIGURE 16-15 Development of a localized Arthus reaction (type III hypersensitive reaction). Complement activation initiated by immune complexes (classical pathway) produces complement intermediates that (1) mediate mast-cell degranulation, (2) chemotactically attract neutrophils, and (3) stimulate release of lytic enzymes from neutrophils trying to phagocytose C_3b-coated immune complexes.

reactions induced by bacterial spores, fungi, or dried fecal proteins can also cause pneumonitis or alveolitis. These reactions are known by a variety of common names reflecting the source of the antigen. For example, "farmer's lung" develops after inhalation of thermophilic actinomycetes from moldy hay, and "pigeon fancier's disease" results from inhalation of a serum protein in dust derived from dried pigeon feces.

Type III Reactions Can Also Be Generalized

When large amounts of antigen enter the bloodstream and bind to antibody, circulating immune complexes can form. If antigen is in excess, small complexes form; because these are not easily cleared by the phagocytic cells, they can cause tissue-damaging type III reactions at various sites. Historically, generalized type III reactions were often observed after the administration of antitoxins containing foreign serum, such as horse antitetanus or antidiphtheria serum. In such cases, the recipient of a foreign antiserum develops antibodies specific for the foreign serum proteins; these antibodies then form circulating immune complexes with the foreign serum antigens. Typically, within days or weeks after exposure to foreign serum antigens, an individual begins to manifest a combination of symptoms that are called **serum sickness** (Figure 16-16). These symptoms include fever, weakness, generalized vasculitis (rashes) with edema and erythema, lymphadenopathy, arthritis, and sometimes glomerulonephritis. The precise manifestations of serum sickness depend on the quantity of immune complexes formed as well as the overall size of the complexes, which determine the site of their deposition. As mentioned above, the sites of deposition vary but, in general, complexes accumulate in tissues where filtration of plasma occurs. This explains the high incidence of glomerulonephritis (complex deposition in the kidney) and vasculitis (deposition in the arteries) and arthritis (deposition in the synovial joints) caused by serum sickness.

Formation of circulating immune complexes contributes to the pathogenesis of a number of conditions other than serum sickness. These include the following:

- *Autoimmune Diseases*
  - Systemic lupus erythematosus
  - Rheumatoid arthritis
  - Goodpasture's syndrome
- *Drug Reactions*
  - Allergies to penicillin and sulfonamides
- *Infectious Diseases*
  - Poststreptococcal glomerulonephritis
  - Meningitis
  - Hepatitis
  - Mononucleosis
  - Malaria
  - Trypanosomiasis

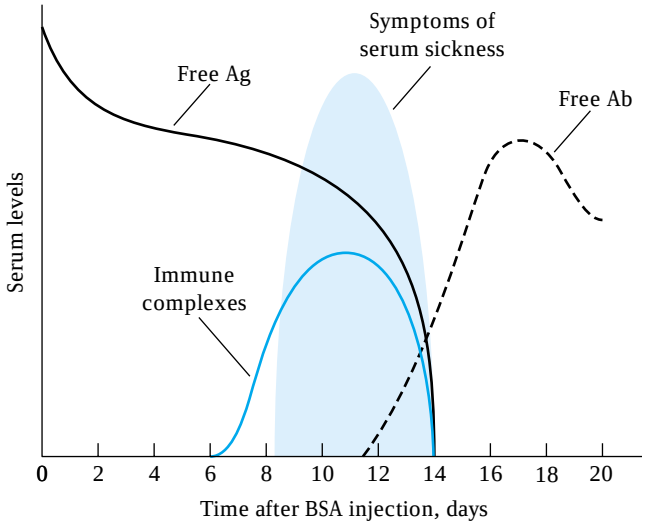


FIGURE 16-16 Correlation between formation of immune complexes and development of symptoms of serum sickness. A large dose of antigen (BSA) was injected into a rabbit at day 0. As antibody formed, it complexed with the antigen and was deposited in the kidneys, joints, and capillaries. The symptoms of serum sickness (light blue area) corresponded to the peak in immune-complex formation. As the immune complexes were cleared, free circulating antibody (dashed black curve) was detected and the symptoms of serum sickness subsided. *[Based on F. G. Germuth, Jr., 1953,* J. Exp. Med. ***97:****257.]*

Complexes of antibody with various bacterial, viral, and parasitic antigens have been shown to induce a variety of type III hypersensitive reactions, including skin rashes, arthritic symptoms, and glomerulonephritis. Poststreptococcal glomerulonephritis, for example, develops when circulating complexes of antibody and streptococcal antigens are deposited in the kidney and damage the glomeruli. A number of autoimmune diseases stem from circulating complexes of antibody with self-proteins, with glycoproteins, or even with DNA. In systemic lupus erythematosus, complexes of DNA and anti-DNA antibodies accumulate in synovial membranes, causing arthritic symptoms, or accumulate on the basement membrane of the kidney, causing progressive kidney damage.

Type IV or Delayed-Type Hypersensitivity (DTH)

When some subpopulations of activated T_H cells encounter certain types of antigens, they secrete cytokines that induce a localized inflammatory reaction called delayed-type hypersensitivity (DTH). The reaction is characterized by large influxes of nonspecific inflammatory cells, in particular, macrophages. This type of reaction was first described in 1890 by

Robert Koch, who observed that individuals infected with *Mycobacterium tuberculosis* developed a localized inflammatory response when injected intradermally with a filtrate derived from a mycobacterial culture. He called this localized skin reaction a "tuberculin reaction." Later, as it became apparent that a variety of other antigens could induce this response (Table 16-6), its name was changed to delayed-type or type IV hypersensitivity in reference to the delayed onset of the reaction and to the tissue damage (hypersensitivity) that is often associated with it. The term *hypersensitivity* is somewhat misleading, for it suggests that a DTH response is always detrimental. Although in some cases a DTH response does cause extensive tissue damage and is in itself pathologic, in many cases tissue damage is limited, and the response plays an important role in defense against intracellular pathogens and contact antigens. The hallmarks of a type IV reaction are the delay in time required for the reaction to develop and the recruitment of macrophages as opposed to neutrophils, as found in a type III reaction. Macrophages are the major component of the infiltrate that surrounds the site of inflammation.

There Are Several Phases of the DTH Response

The development of the DTH response begins with an initial sensitization phase of 1–2 weeks after primary contact with an antigen. During this period, T_H cells are activated and clonally expanded by antigen presented together with the requisite class II MHC molecule on an appropriate antigen-presenting cell (Figure 16-17a). A variety of antigen-presenting cells have been shown to be involved in the activation of a DTH response, including Langerhans cells and macrophages. Langerhans cells are dendritic cells found in the epidermis. These cells are thought to pick up antigen that enters through the skin and transport it to regional lymph nodes, where T cells are activated by the antigen. In some species, including humans, the vascular endothelial cells express class II MHC molecules and also function as antigen-presenting cells in the development of the DTH response. Generally, the T cells activated during the sensitization phase are $CD4^+$, primarily of the T_H1 subtype, but in a few cases $CD8^+$ cells have also been shown to induce a DTH response. The activated T cells previously were called T_{DTH} cells to denote their function in the DTH response, although in reality they are simply a subset of activated T_H1 cells (or, in some cases, T_C cells).

A subsequent exposure to the antigen induces the effector phase of the DTH response (see Figure 16-17b). In the effector phase, T_H1 cells secrete a variety of cytokines that recruit and activate macrophages and other nonspecific inflammatory cells. A DTH response normally does not become apparent until an average of 24 h after the second contact with the antigen; the response generally peaks 48–72 h after second contact. The delayed onset of this response reflects the time required for the cytokines to induce localized influxes of macrophages and their activation. Once a DTH response begins, a complex interplay of nonspecific cells and mediators is set in motion that can result in tremendous amplification. By the time the DTH response is fully developed, only about 5% of the participating cells are antigen-specific T_H1 cells; the remainder are macrophages and other nonspecific cells.

Macrophages are the principal effector cells of the DTH response. Cytokines elaborated by T_H1 cells induce blood monocytes to adhere to vascular endothelial cells and migrate from the blood into the surrounding tissues. During this process the monocytes differentiate into activated macrophages. As Chapter 2 described, activated macrophages exhibit increased levels of phagocytosis and an increased ability to kill microorganisms through various cytotoxic mediators. In addition, activated macrophages express increased levels of class II MHC molecules and cell-adhesion molecules and therefore function more effectively as antigen-presenting cells.

The influx and activation of macrophages in the DTH response is important in host defense against parasites and bacteria that live within cells, where circulating antibodies cannot reach them. The heightened phagocytic activity and the buildup of lytic enzymes from macrophages in the area of infection lead to nonspecific destruction of cells, and thus of the intracellular pathogen. Generally, the pathogen is cleared rapidly with little tissue damage. However, in some cases, especially if the antigen is not easily cleared, a prolonged DTH response can itself become destructive to the host as the intense inflammatory response develops into a visible granulomatous reaction. A granuloma develops when continuous activation of macrophages induces the macrophages to adhere closely to one another, assuming an epithelioid shape and sometimes fusing to form multinucleated giant cells (Figure 16-18). These giant cells displace the normal tissue cells, forming palpable nodules, and release high concentrations of lytic enzymes, which destroy surrounding tissue. In these cases, the response can damage blood vessels and lead

TABLE 16-6 Intracellular pathogens and contact antigens that induce delayed-type (type IV) hypersensitivity

Intracellular bacteria	Intracellular viruses
Mycobacterium tuberculosis	Herpes simplex virus
Mycobacterium leprae	Variola (smallpox)
Listeria monocytogenes	Measles virus
Brucella abortus	
Intracellular fungi	Contact antigens
Pneumocystis carinii	Picrylchloride
Candida albicans	Hair dyes
Histoplasma capsulatum	Nickel salts
Cryptococcus neoformans	Poison ivy
Intracellular parasites	Poison oak
Leishmania sp.	

VISUALIZING CONCEPTS

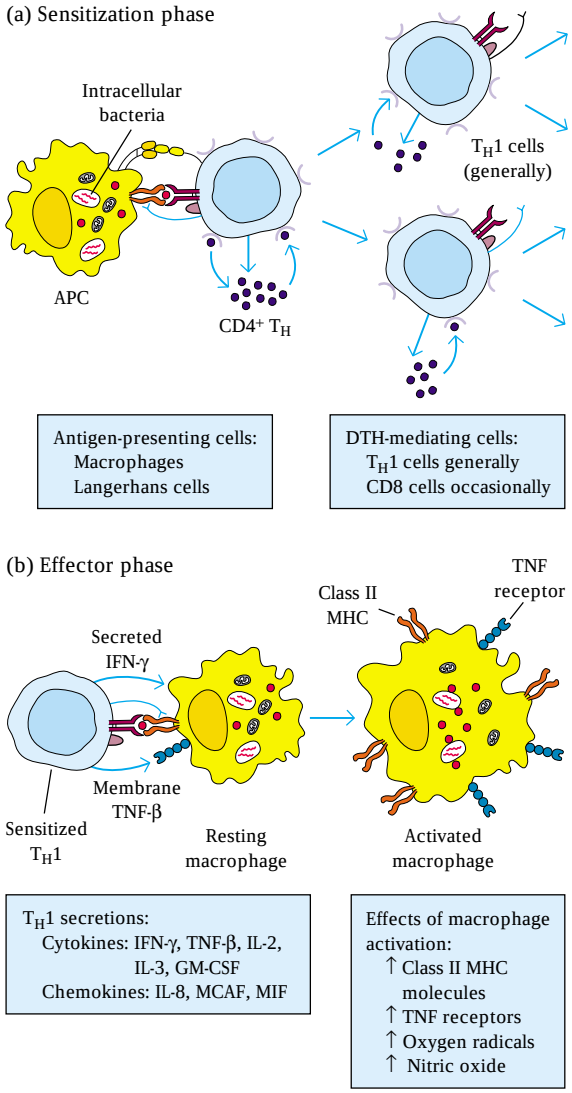


FIGURE 16-17 Overview of the DTH response. (a) In the sensitization phase after initial contact with antigen (e.g., peptides derived from intracellular bacteria), T_H cells proliferate and differentiate into T_H1 cells. Cytokines secreted by these T cells are indicated by the dark blue balls. (b) In the effector phase after subsequent exposure of sensitized T_H1 cells to antigen, the T_H1 cells secrete a variety of cytokines and chemokines. These factors attract and activate macrophages and other nonspecific inflammatory cells. Activated macrophages are more effective in presenting antigen, thus perpetuating the DTH response, and function as the primary effector cells in this reaction.

to extensive tissue necrosis. The response to *Mycobacterium tuberculosis* illustrates the double-edged nature of the DTH response. Immunity to this intracellular bacterium involves a DTH response in which activated macrophages wall off the organism in the lung and contain it within a granuloma-type lesion called a tubercle. Often, however, the concentrated release of lytic enzymes from the activated macrophages within tubercles damages lung tissue. Some examples of truly hypersensitive conditions, in which tissue damage far outweighs any beneficial effects, are described in Chapter 17.

Numerous Cytokines Participate in the DTH Reaction

Among the cytokines produced by T_H1 cells are a number that attract and activate macrophages to the site of infection. IL-3 and GM-CSF induce localized hematopoiesis of the granulocyte-monocyte lineage. IFN-γ and TNF-β (together with macrophage-derived TNF-α and IL-1) act on nearby endothelial cells, inducing a number of changes that facilitate extravasation of monocytes and other nonspecific inflam-

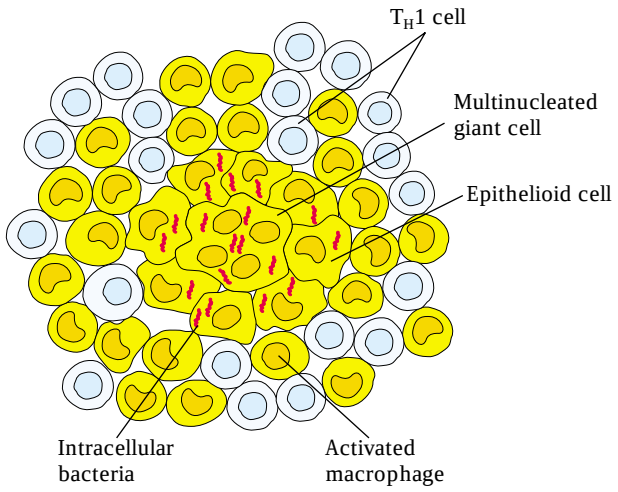


FIGURE 16-18 A prolonged DTH response can lead to formation of a granuloma, a nodule-like mass. Lytic enzymes released from activated macrophages in a granuloma can cause extensive tissue damage.

matory cells. Circulating neutrophils and monocytes adhere to the adhesion molecules displayed on the vascular endothelial cells and extravasate into the tissue spaces. Neutrophils appear early in the reaction, peaking by about 6 h and then declining in numbers. The monocyte infiltration occurs between 24 and 48 h after antigen exposure.

As the monocytes enter the tissues to become macrophages, they are chemotactically drawn to the site of the DTH response by chemokines such as monocyte chemotactic and activating factor (MCAF). Another chemokine called migration-inhibition factor (MIF) inhibits macrophages from migrating beyond the site of a DTH reaction. As macrophages accumulate at the site of a DTH reaction, they are activated by cytokines, particularly IFN-γ and membrane-bound TNF-β produced by T_H1 cells. As noted earlier, macrophages become more effective as antigen-presenting cells upon activation. Thus, the activated macrophages can efficiently mediate activation of more T cells, which in turn secrete more cytokines that recruit and activate even more macrophages. This self-perpetuating response, however, is a double-edged sword, with a fine line existing between a beneficial, protective response and a detrimental response characterized by extensive tissue damage.

A report of experiments with knockout mice that could not produce IFN-γ demonstrated the importance of this cytokine in the DTH response. When these knockout mice were infected with an attenuated strain of *Mycobacterium bovis* known as BCG (Bacille Calmette Guérin), nearly all the animals died within 60 days, whereas wild-type mice survived (Figure 16-19). Macrophages from the IFN-γ knockout mice were shown to have reduced levels of class II MHC molecules and of bactericidal metabolites such as nitric oxide and superoxide anion.

The DTH Reaction Is Detected with a Skin Test

The presence of a DTH reaction can be measured experimentally by injecting antigen intradermally into an animal and observing whether a characteristic skin lesion develops at the injection site. A positive skin-test reaction indicates that the individual has a population of sensitized T_H1 cells specific for the test antigen. For example, to determine whether an individual has been exposed to *M. tuberculosis*, PPD, a protein derived from the cell wall of this mycobacterium, is injected intradermally. Development of a red, slightly swollen, firm lesion at the site between 48 and 72 h later indicates previous exposure. The skin lesion results from intense infiltration of cells to the site of injection during a DTH reaction; 80%–90% of these cells are macrophages. Note, however, that a positive test does not allow one to conclude whether the exposure was to a pathogenic form of *M. tuberculosis* or to a vaccine form received through immunization, which is performed in some parts of the world.

Contact Dermatitis Is a Type of DTH Response

Many contact-dermatitis reactions, including the responses to formaldehyde, trinitrophenol, nickel, turpentine, and active agents in various cosmetics and hair dyes, poison oak, and poison ivy, are mediated by T_H1 cells. Most of these substances are small molecules that can complex with skin proteins.

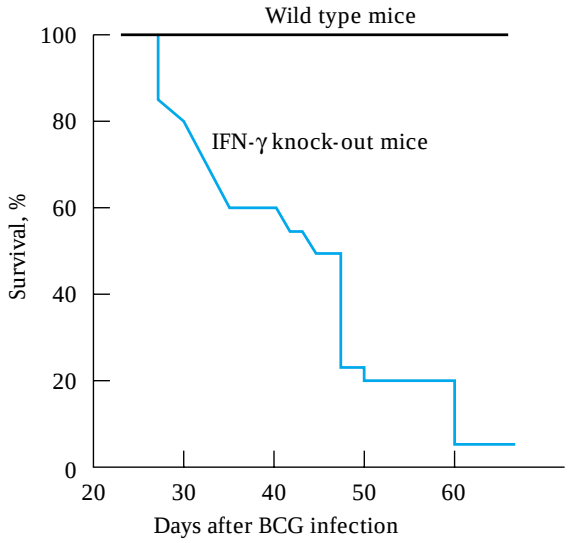


FIGURE 16-19 Experimental demonstration of the role of IFN-γ in host defense against intracellular pathogens. Knockout mice were produced by introducing a targeted mutation in the gene encoding IFN-γ. The mice were then infected with 10^7 colony-forming units of attenuated *Mycobacterium bovis* (BCG) and their survival monitored. *[Adapted from D. K. Dalton et al., 1993,* Science ***259:****1739.]*

This complex is internalized by antigen-presenting cells in the skin (e.g., Langerhans cells), then processed and presented together with class II MHC molecules, causing activation of sensitized T_H1 cells. In the reaction to poison oak, for example, a pentadecacatechol compound from the leaves of the plant forms a complex with skin proteins. When T_H cells react with this compound appropriately displayed by local antigen-presenting cells, they differentiate into sensitized T_H1 cells. A subsequent exposure to pentadecacatechol will elicit activation of T_H1 cells and induce cytokine production (Figure 16-20). Approximately 48–72 h after the second exposure, the secreted cytokines cause macrophages to accumulate at the site. Activation of these macrophages and release of lytic enzymes result in the redness and pustules that characterize a reaction to poison oak.

SUMMARY

- Hypersensitive reactions are inflammatory reactions within the humoral or cell-mediated branches of the immune system that lead to extensive tissue damage or even death. The four types of hypersensitive reaction generate characteristic effector molecules and clinical manifestations.
- A type I hypersensitive reaction is mediated by IgE antibodies, whose Fc region binds to receptors on mast cells or blood basophils. Crosslinkage of the fixed IgE by allergen leads to mast cell or basophil degranulation with release of pharmacologically active mediators. The principal effects of these mediators are smooth-muscle contraction and vasodilation. Clinical manifestations of type I reactions include potentially life-threatening systemic anaphylaxis and localized responses such as hay fever and asthma.
- A type II hypersensitive reaction occurs when antibody reacts with antigenic determinants present on the surface of cells, leading to cell damage or death through complement-mediated lysis or antibody-dependent cell-mediated cytotoxicity (ADCC). Transfusion reactions and hemolytic disease of the newborn are type II reactions.
- A type III hypersensitive reaction is mediated by the formation of immune complexes and the ensuing activation of complement. Complement split products serve as immune effector molecules that elicit localized vasodilation and chemotactically attract neutrophils. Deposition of immune complexes near the site of antigen entry can induce an Arthus reaction, in which lytic enzymes released by the accumulated neutrophils and the complement membrane-attack complex cause localized tissue damage.
- A type IV hypersensitive reaction involves the cell-mediated branch of the immune system. Antigen activation of sensitized T_H1 cells induces release of various cytokines that cause macrophages to accumulate and become activated. The net effect of the activation of macrophages is to release lytic enzymes that cause localized tissue damage.

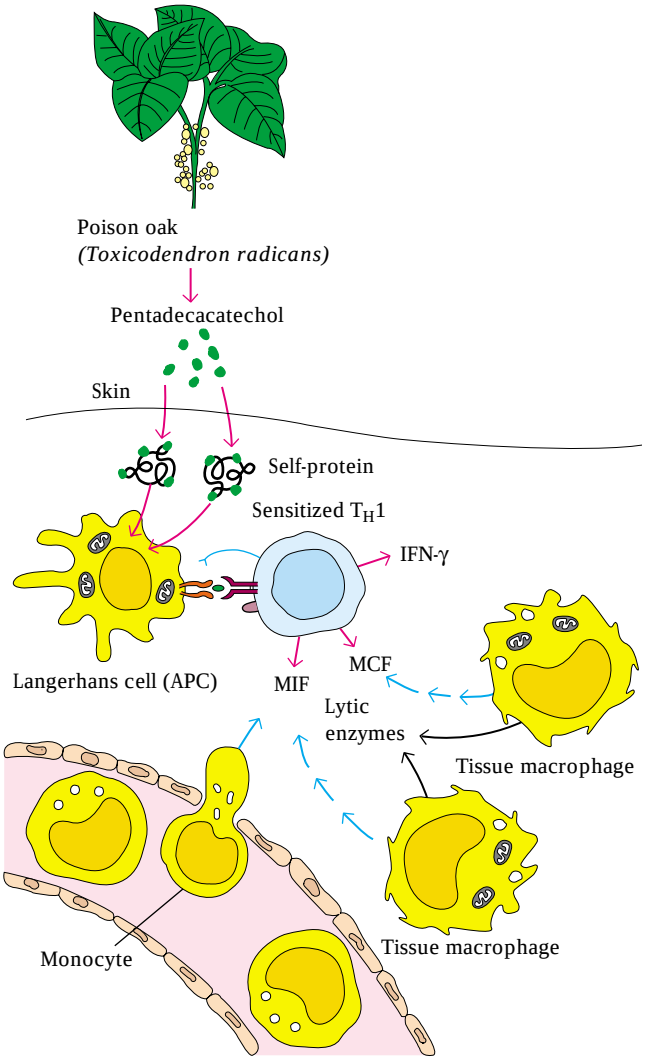


FIGURE 16-20 Development of delayed-type hypersensitivity reaction after a second exposure to poison oak. Cytokines such as IFN-γ, macrophage-chemotactic factor (MCF), and migration-inhibition factor (MIF) released from sensitized T_H1 cells mediate this reaction. Tissue damage results from lytic enzymes released from activated macrophages.

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AU: "D.G." or "D.W."?

USEFUL WEB SITES

http://www.niaid.nih.gov/

National Institute of Allergy and Infectious Diseases homepage. NIAID is the NIH Institute that sponsors research in infectious diseases. Their Web site provides a number of links to other relevant sites.

http://allergy.mcg.edu/home.html

A site maintained by the American College of Allergy, Asthma & Immunology. An excellent source of patient information about many allergies. This site contains many valuable links.

http//:www.glaxowellcome.co.uk/health/actiontb/

Action TB—A review of tuberculosis for a general audience that appears on the Glaxo-Wellcome company's Web site.

http//:www.aaaai.org/

The American Association of Allergy, Asthma and Immunology Web site. A good site for exploring the many aspects of asthma.

Study Questions

CLINICAL FOCUS QUESTION Discuss why IL-4 and FcεRIβ are excellent candidate genes involved in the genetic susceptibility to asthma.

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

   a. Mice infected with *Nippostrongylus brasiliensis* exhibit decreased production of IgE.
   b. IL-4 decreases IgE production by B cells.
   c. The initial step in the process of mast-cell degranulation is crosslinking of Fc receptors.
   d. Antihistamines are effective for the treatment of type III hypersensitivity.
   e. Most pollen allergens contain a single allergenic component.
   f. Babies can acquire IgE-mediated allergies by passive transfer of maternal antibody.
   g. Transfusion reactions are a manifestation of type II hypersensitivity.

2. In an immunology laboratory exercise, you are studying the response of mice injected intradermally with complete antibodies to the IgE Fc receptor (FcεR1) or with Fab fragments of such antibodies.

   a. Predict the response expected with each type of antibody.
   b. Would the responses observed depend on whether the mice were allergic? Explain.

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Review and quiz of key terms

3. Serum sickness can result when an individual is given a large dose of antiserum such as a mouse antitoxin to snake venom. How could you take advantage of recent technological advances to produce an antitoxin that would not produce serum sickness in patients who receive it?

4. What immunologic mechanisms most likely account for a person's developing each of the following reactions after an insect bite?

a. Within 1–2 min after being bitten, swelling and redness appear at the site and then disappear by 1 h.
b. 6–8 h later, swelling and redness again appear and persist for 24 h.
c. 72 h later, the tissue becomes inflamed, and tissue necrosis follows.

5. Indicate which type(s) of hypersensitive reaction (I–IV) apply to the following characteristics. Each characteristic can apply to one, or more than one, type.

a. Is an important defense against intracellular pathogens.
b. Can be induced by penicillin.
c. Involves histamine as an important mediator.
d. Can be induced by poison oak in sensitive individuals.
e. Can lead to symptoms of asthma.
f. Occurs as result of mismatched blood transfusion.
g. Systemic form of reaction is treated with epinephrine.
h. Can be induced by pollens and certain foods in sensitive individuals.
i. May involve cell destruction by antibody-dependent cell-mediated cytotoxicity.
j. One form of clinical manifestation is prevented by Rhogam.
k. Localized form characterized by wheal and flare reaction.

6. In the table below, indicate whether each immunologic event listed does (+) or does not (–) occur in each type of hypersensitive response.

	Hypersensitivity			
Immunologic event	Type I	Type II	Type III	Type IV
IgE-mediated degranulation of mast cells				
Lysis of antibody-coated blood cells by complement				
Tissue destruction in response to poison oak				
C3a- and C5a-mediated mast-cell degranulation				
Chemotaxis of neutrophils				
Chemotaxis of eosinophils				
Activation of macrophages by IFN-γ				
Deposition of antigen-antibody complexes on basement membranes of capillaries				
Sudden death due to vascular collapse (shock) shortly after injection or ingestion of antigen				