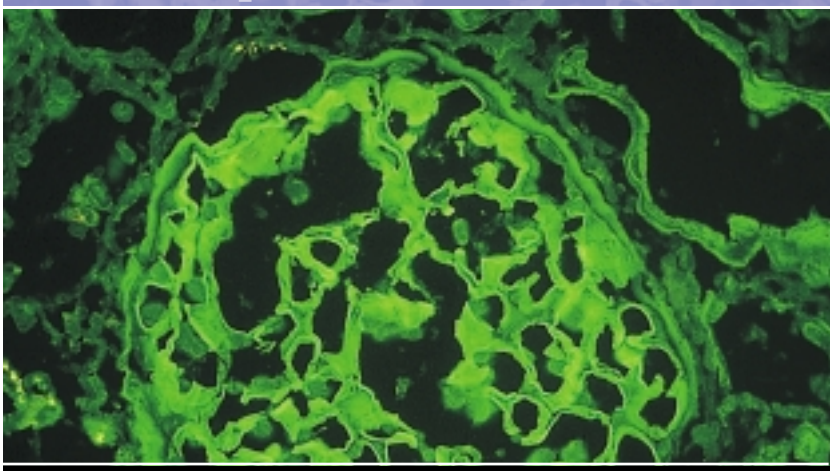
chapter 20

Autoimmunity



Kidney Biopsy from Goodpasture's Syndrone

Early in the last century, Paul Ehrlich realized that the immune system could go awry and, instead of reacting against foreign antigens, could focus its attack on self-antigens. He termed this condition "horror autotoxicus." We now understand that, while mechanisms of self-tolerance normally protect an individual from potentially self-reactive lymphocytes, there are failures. They result in an inappropriate response of the immune system against self-components termed **autoimmunity.** In the 1960s, it was believed that all self-reactive lymphocytes were eliminated during their development in the bone marrow and thymus and that a failure to eliminate these lymphocytes led to autoimmune consequences. Since the late 1970s, a broad body of experimental evidence has countered that belief, revealing that not all self-reactive lymphocytes are deleted during T-cell and B-cell maturation. Instead, normal healthy individuals have been shown to possess mature, recirculating, self-reactive lymphocytes. Since the presence of these self-reactive lymphocytes in the periphery does not inevitably result in autoimmune reactions, their activity must be regulated in normal individuals through clonal anergy or clonal suppression. A breakdown in this regulation can lead to activation of self-reactive clones of T or B cells, generating humoral or cell-mediated responses against self-antigens. These reactions can cause serious damage to cells and organs, sometimes with fatal consequences.

Sometimes the damage to self-cells or organs is caused by antibodies; in other cases, T cells are the culprit. For example, a common form of autoimmunity is tissue injury by mechanisms similar to type II hypersensitivity reactions. As Chapter 16 showed, type II hypersensitivity reactions involve antibody-mediated destruction of cells. Autoimmune hemolytic anemia is an excellent example of such an autoimmune disease. In this disease, antigens on red blood cells are recognized by auto-antibodies, which results in the destruction of the blood cells, which in turn results in anemia. Auto-antibodies are also the major offender in Hashimoto's thyroiditis, in which antibodies reactive with tissue-specific antigens such as thyroid peroxidase and thyroglobulin cause severe tissue destruction. Other autoimmune diseases that involve auto-antibodies are listed in Table 20-1.

Many autoimmune diseases are characterized by tissue destruction mediated directly by T cells. A well-known example is rheumatoid arthritis, in which self-reactive T cells attack the tissue in joints, causing an inflammatory response that results in swelling and tissue destruction. Other examples include insulin-dependent diabetes mellitus and multiple sclerosis (see Table 20-1).

This chapter describes some common human autoimmune diseases. These can be divided into two broad categories: organ-specific and systemic autoimmune disease (Table 20-1). Such diseases affect 5%–7% of the human population, often causing chronic debilitating illnesses. Several experimental animal models used to study autoimmunity and various mechanisms that may contribute to induction of autoimmune reactions also are described. Finally, current and experimental therapies for treating autoimmune diseases are described.

Organ-Specific Autoimmune Diseases

In an organ-specific autoimmune disease, the immune response is directed to a target antigen unique to a single organ or gland, so that the manifestations are largely limited to that organ. The cells of the target organs may be damaged di-

TABLE 20-1 Some autoimmune diseases in humans

Disease	Self-antigen	Immune response
	ORGAN-SPECIFIC AUTOIMMUNE DISEASES	
Addison's disease	Adrenal cells	Auto-antibodies
Autoimmune hemolytic anemia	RBC membrane proteins	Auto-antibodies
Goodpasture's syndrome	Renal and lung basement membranes	Auto-antibodies
Graves' disease	Thyroid-stimulating hormone receptor	Auto-antibody (stimulating)
Hashimoto's thyroiditis	Thyroid proteins and cells	T_{DTH} cells, auto-antibodies
Idiopathic thrombocyopenia purpura	Platelet membrane proteins	Auto-antibodies
Insulin-dependent diabetes mellitus	Pancreatic beta cells	T_{DTH} cells, auto-antibodies
Myasthenia gravis	Acetylcholine receptors	Auto-antibody (blocking)
Myocardial infarction	Heart	Auto-antibodies
Pernicious anemia	Gastric parietal cells; intrinsic factor	Auto-antibody
Poststreptococcal glomerulonephritis	Kidney	Antigen-antibody complexes
Spontaneous infertility	Sperm	Auto-antibodies
	SYSTEMIC AUTOIMMUNE DISEASES	
Ankylosing sponkylitis	Vertebrae	Immune complexes
Multiple sclerosis	Brain or white matter	T_H1 cells and T_C cells, auto-antibodies
Rheumatoid arthritis	Connective tissue, IgG	Auto-antibodies, immune complexes
Scleroderma	Nuclei, heart, lungs, gastrointestinal tract, kidney	Auto-antibodies
Sjogren's syndrome	Salivary gland, liver, kidney, thyroid	Auto-antibodies
Systemic lupus erythematosus (SLE)	DNA, nuclear protein, RBC and platelet membranes	Auto-antibodies, immune complexes

rectly by humoral or cell-mediated effector mechanisms. Alternatively, the antibodies may overstimulate or block the normal function of the target organ.

Some Autoimmune Diseases Are Mediated by Direct Cellular Damage

Autoimmune diseases involving direct cellular damage occur when lymphocytes or antibodies bind to cell-membrane antigens, causing cellular lysis and/or an inflammatory response in the affected organ. Gradually, the damaged cellular structure is replaced by connective tissue (scar tissue), and the function of the organ declines. This section briefly describes a few examples of this type of autoimmune disease.

HASHIMOTO'S THYROIDITIS

In Hashimoto's thyroiditis, which is most frequently seen in middle-aged women, an individual produces auto-antibodies and sensitized T_H1 cells specific for thyroid antigens. The DTH response is characterized by an intense infiltration of the thyroid gland by lymphocytes, macrophages, and plasma cells, which form lymphocytic follicles and germinal centers (Figure 20-1). The ensuing inflammatory response causes a goiter, or visible enlargement of the thyroid gland, a physiological response to hypothyroidism. Antibodies are formed to a number of thyroid proteins, including thyroglobulin and thyroid peroxidase, both of which are involved in the uptake of iodine. Binding of the auto-antibodies to these proteins interferes with iodine uptake and leads to decreased production of thyroid hormones (hypothyroidism).

AUTOIMMUNE ANEMIAS

Autoimmune anemias include pernicious anemia, autoimmune hemolytic anemia, and drug-induced hemolytic anemia. Pernicious anemia is caused by auto-antibodies to intrinsic factor, a membrane-bound intestinal protein on gastric parietal cells. Intrinsic factor facilitates uptake of vitamin B_{12}

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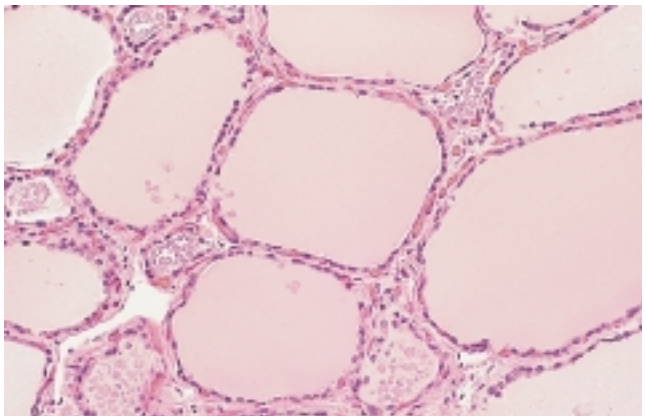

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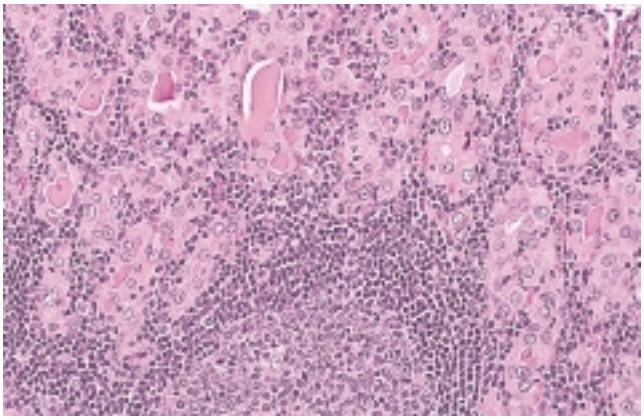

FIGURE 20-1 Photomicrographs of (a) normal thyroid gland showing a follicle lined by cuboidal follicular epithelial cells and (b) gland in Hashimoto's thyroiditis showing intense lymphocyte infiltration. *[From Web Path, courtesy of E. C. Klatt, University of Utah.]*

from the small intestine. Binding of the auto-antibody to intrinsic factor blocks the intrinsic factor–mediated absorption of vitamin B_{12}. In the absence of sufficient vitamin B_{12}, which is necessary for proper hematopoiesis, the number of functional mature red blood cells decreases below normal. Pernicious anemia is treated with injections of vitamin B_{12}, thus circumventing the defect in its absorption.

An individual with autoimmune hemolytic anemia makes auto-antibody to RBC antigens, triggering complement-mediated lysis or antibody-mediated opsonization and phagocytosis of the red blood cells. One form of autoimmune anemia is drug-induced: when certain drugs such as penicillin or the anti-hypertensive agent methyldopa interact with red blood cells, the cells become antigenic. The immunodiagnostic test for autoimmune hemolytic anemias generally involves a Coombs test, in which the red cells are incubated with an anti–human IgG antiserum. If IgG auto-antibodies are present on the red cells, the cells are agglutinated by the antiserum.

GOODPASTURE'S SYNDROME

In **Goodpasture's syndrome,** auto-antibodies specific for certain basement-membrane antigens bind to the basement membranes of the kidney glomeruli and the alveoli of the lungs. Subsequent complement activation leads to direct cellular damage and an ensuing inflammatory response mediated by a buildup of complement split products. Damage to the glomerular and alveolar basement membranes leads to progressive kidney damage and pulmonary hemorrhage. Death may ensue within several months of the onset of symptoms. Biopsies from patients with Goodpasture's syndrome stained with fluorescent-labeled anti-IgG and anti-C3b reveal linear deposits of IgG and C3b along the basement membranes (Figure 20-2).

INSULIN-DEPENDENT DIABETES MELLITUS

A disease afflicting 0.2% of the population, **insulin-dependent diabetes mellitus (IDDM)** is caused by an autoimmune attack on the pancreas. The attack is directed against specialized insulin-producing cells (beta cells) that are located in spherical clusters, called the islets of Langerhans, scattered throughout the pancreas. The autoimmune attack destroys beta cells, resulting in decreased production of insulin and consequently increased levels of blood glucose. Several factors are important in the destruction of beta cells. First, activated CTLs migrate into an islet and begin to attack the insulin-producing cells. Local cytokine production during this

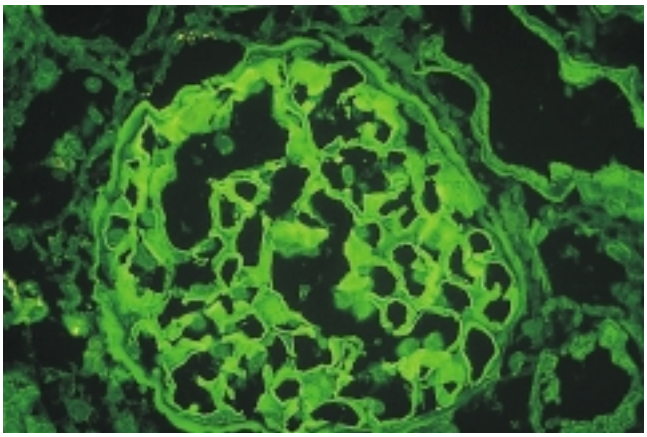

FIGURE 20-2 Fluorescent anti-IgG staining of a kidney biopsy from a patient with Goodpasture's syndrome reveals linear deposits of auto-antibody along the basement membrane. *[From Web Path, courtesy of E. C. Klatt, University of Utah.]*

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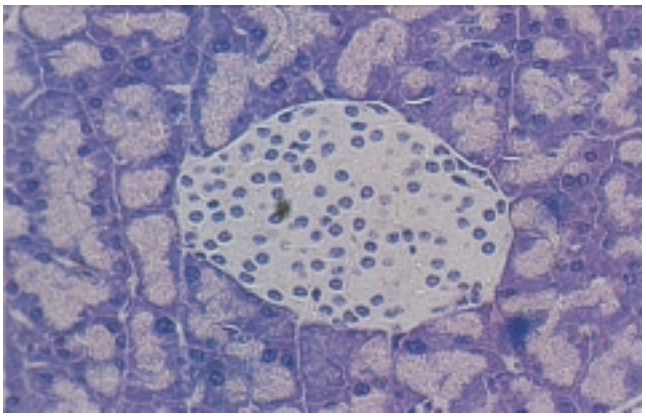

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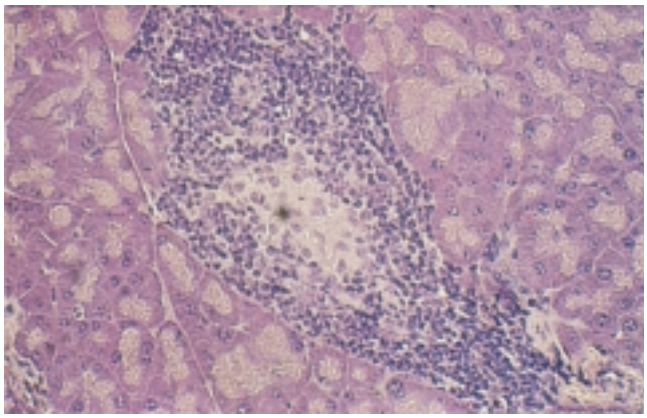

FIGURE 20-3 Photomicrographs of an islet of Langerhans (a) in pancreas from a normal mouse and (b) one in pancreas from a mouse with a disease resembling insulin-dependent diabetes mellitus. Note the lymphocyte infiltration into the islet (insulitis) in (b). *[From M. A. Atkinson and N. K. Maclaren, 1990,* Sci. Am. ***263****(1):62.]*

response includes IFN-γ, TNF-α, and IL-1. Auto-antibody production can also be a contributing factor in IDDM. The first CTL infiltration and activation of macrophages, frequently referred to as insulitis (Figure 20-3), is followed by cytokine release and the presence of auto-antibodies, which leads to a cell-mediated DTH response. The subsequent beta-cell destruction is thought to be mediated by cytokines released during the DTH response and by lytic enzymes released from the activated macrophages. Auto-antibodies to beta cells may contribute to cell destruction by facilitating either antibody-plus-complement lysis or antibody-dependent cell-mediated cytotoxicity (ADCC).

The abnormalities in glucose metabolism that are caused by the destruction of islet beta cells result in serious metabolic problems that include ketoacidosis and increased urine production. The late stages of the disease are often characterized by atherosclerotic vascular lesions—which in turn cause gangrene of the extremities due to impeded vascular flow—renal failure, and blindness. If untreated, death can result. The most common therapy for diabetes is daily administration of insulin. This is quite helpful in managing the disease, but, because sporadic doses are not the same as metabolically regulated continuous and controlled release of the hormone, periodically injected doses of insulin do not totally alleviate the problems caused by the disease. Another complicating feature of diabetes is that the disorder can go undetected for several years, allowing irreparable loss of pancreatic tissue to occur before treatment begins.

Some Autoimmune Diseases Are Mediated by Stimulating or Blocking Auto-Antibodies

In some autoimmune diseases, antibodies act as agonists, binding to hormone receptors in lieu of the normal ligand and stimulating inappropriate activity. This usually leads to an overproduction of mediators or an increase in cell growth. Conversely, auto-antibodies may act as antagonists, binding hormone receptors but blocking receptor function. This generally causes impaired secretion of mediators and gradual atrophy of the affected organ.

GRAVES' DISEASE

The production of thyroid hormones is carefully regulated by thyroid-stimulating hormone (TSH), which is produced by the pituitary gland. Binding of TSH to a receptor on thyroid cells activates adenylate cyclase and stimulates the synthesis of two thyroid hormones, thyroxine and triiodothyronine. A patient with **Graves' disease** produces auto-antibodies that bind the receptor for TSH and mimic the normal action of TSH, activating adenylate cyclase and resulting in production of the thyroid hormones. Unlike TSH, however, the auto-antibodies are not regulated, and consequently they over-stimulate the thyroid. For this reason these auto-antibodies are called long-acting thyroid-stimulating (LATS) antibodies (Figure 20-4).

MYASTHENIA GRAVIS

Myasthenia gravis is the prototype autoimmune disease mediated by blocking antibodies. A patient with this disease produces auto-antibodies that bind the acetylcholine receptors on the motor end-plates of muscles, blocking the normal binding of acetylcholine and also inducing complement-mediated lysis of the cells. The result is a progressive weakening of the skeletal muscles (Figure 20-5). Ultimately, the antibodies destroy the cells bearing the receptors. The early signs of this disease include drooping eyelids and inability to retract the corners of the mouth, which gives the appearance of snarling. Without treatment, progressive weakening of the

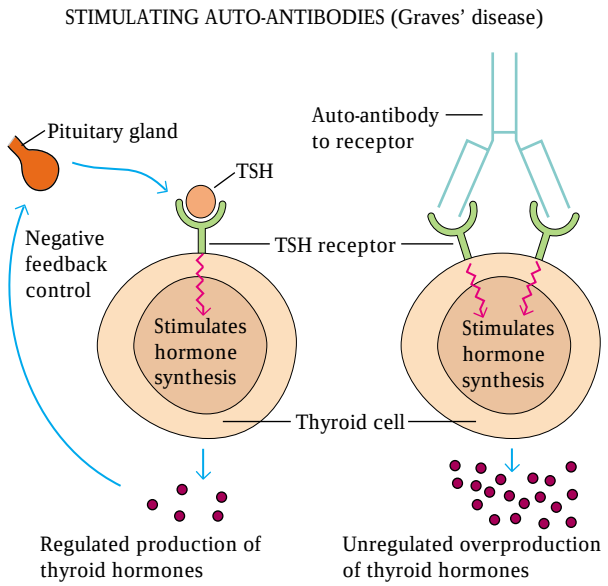

FIGURE 20-4 In Graves' disease, binding of auto-antibodies to the receptor for thyroid-stimulating hormone (TSH) induces unregulated activation of the thyroid, leading to overproduction of the thyroid hormones (purple dots).

muscles can lead to severe impairment of eating as well as problems with movement. However, with appropriate treatment, this disease can be managed quite well and afflicted individuals can lead a normal life.

Systemic Autoimmune Diseases

In systemic autoimmune diseases, the response is directed toward a broad range of target antigens and involves a number of organs and tissues. These diseases reflect a general defect in immune regulation that results in hyperactive T cells and B cells. Tissue damage is widespread, both from cell-mediated immune responses and from direct cellular damage caused by auto-antibodies or by accumulation of immune complexes.

Systemic Lupus Erythematosus Attacks Many Tissues

One of the best examples of a systemic autoimmune disease is **systemic lupus erythematosus (SLE),** which typically appears in women between 20 and 40 years of age; the ratio of female to male patients is 10:1. SLE is characterized by fever, weakness, arthritis, skin rashes, pleurisy, and kidney dysfunction (Figure 20-6). Lupus is more frequent in African-American and Hispanic women than in Caucasians, although it is not known why this is so. Affected individuals may produce auto-antibodies to a vast array of tissue antigens, such as DNA, histones, RBCs, platelets, leukocytes, and clotting factors; interaction of these auto-antibodies with their specific antigens produces various symptoms. Auto-antibody specific for RBCs and platelets, for example, can lead to complement-mediated lysis, resulting in hemolytic anemia and thrombocytopenia, respectively. When immune complexes of auto-antibodies with various nuclear antigens are deposited along the walls of

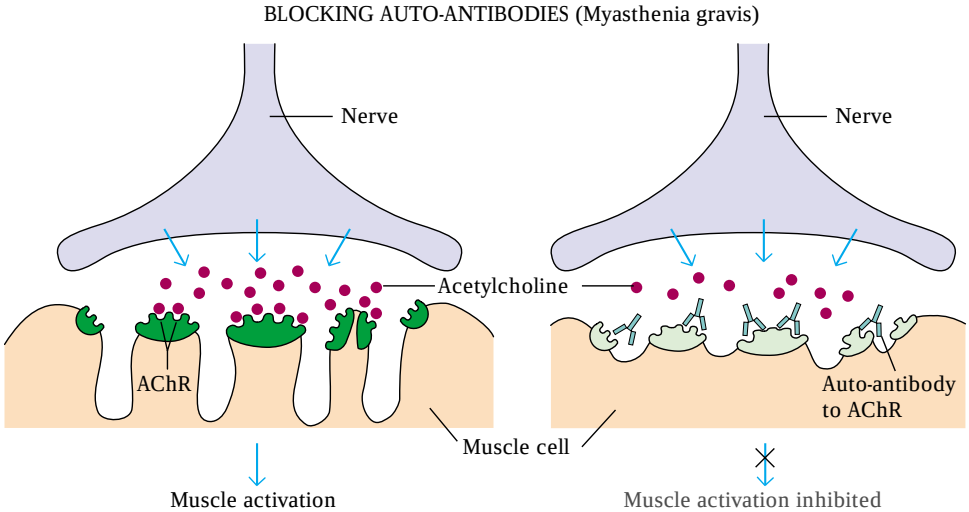

FIGURE 20-5 In myasthenia gravis, binding of auto-antibodies to the acetylcholine receptor (*right*) blocks the normal binding of acetylcholine (burgandy dots) and subsequent muscle activation (*left*). In addition, the anti-AChR auto-antibody activates complement, which damages the muscle end-plate; the number of acetylcholine receptors declines as the disease progresses. AChR = acetylcholine receptor.

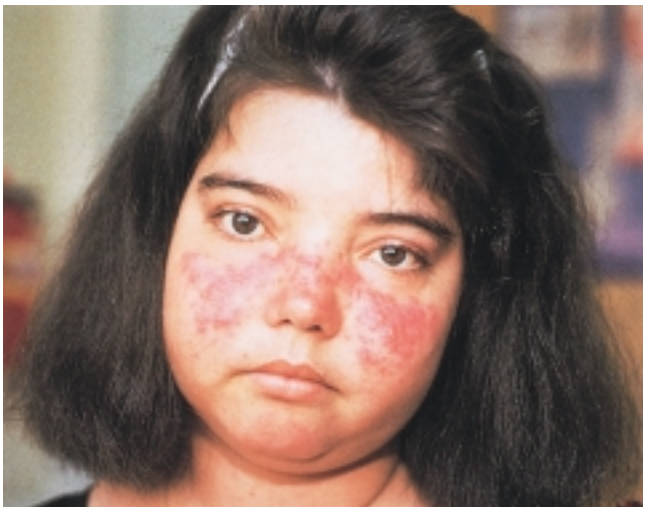

FIGURE 20-6 Characteristic "butterfly" rash over the cheeks of a young girl with systemic lupus erythematosus. *[From L. Steinman, 1993,* Sci. Am. ***269****(3):80.]*

small blood vessels, a type III hypersensitive reaction develops. The complexes activate the complement system and generate membrane-attack complexes and complement split products that damage the wall of the blood vessel, resulting in vasculitis and glomerulonephritis.

Excessive complement activation in patients with severe SLE produces elevated serum levels of the complement split products C3a and C5a, which may be three to four times higher than normal. C5a induces increased expression of the type 3 complement receptor (CR3) on neutrophils, facilitating neutrophil aggregation and attachment to the vascular endothelium. As neutrophils attach to small blood vessels, the number of circulating neutrophils declines (neutropenia) and various occlusions of the small blood vessels develop (vasculitis). These occlusions can lead to widespread tissue damage.

Laboratory diagnosis of SLE focuses on the characteristic antinuclear antibodies, which are directed against double-stranded or single-stranded DNA, nucleoprotein, histones, and nucleolar RNA. Indirect immunofluorescent staining with serum from SLE patients produces various characteristic nucleus-staining patterns.

Multiple Sclerosis Attacks the Central Nervous System

Multiple sclerosis (MS) is the most common cause of neurologic disability associated with disease in Western countries. The symptoms may be mild, such as numbness in the limbs, or severe, such as paralysis or loss of vision. Most people with MS are diagnosed between the ages of 20 and 40. Individuals with this disease produce autoreactive T cells that participate in the formation of inflammatory lesions along the myelin sheath of nerve fibers. The cerebrospinal fluid of patients with active MS contains activated T lymphocytes, which infiltrate the brain tissue and cause characteristic inflammatory lesions, destroying the myelin. Since myelin functions to insulate the nerve fibers, a breakdown in the myelin sheath leads to numerous neurologic dysfunctions.

Epidemiological studies indicate that MS is most common in the Northern hemisphere and, interestingly, in the United States. Populations who live north of the 37th parallel have a prevalence of 110–140 cases per 100,000, while those who live south of the 37th parallel show a prevalence of 57–78 per 100,000. And individuals from south of the 37th parallel who move north assume a new risk if the move occurs before 15 years of age. These provocative data suggest that there is an environmental component of the risk of contracting MS. This is not the entire story, however, since genetic influences also are important. While the average person in the United States has about one chance in 1000 of developing MS, close relatives of people with MS, such as children or siblings, have 1 chance in 50 to 100 of developing MS. The identical twin of a person with MS has a 1 in 3 chance of developing the disease. These data point strongly to the genetic component of the disease. And, as is described in the Clinical Focus of this chapter, MS affects women two to three times more frequently than men.

The cause of MS, like most autoimmune diseases, is not well understood. However, there are some suggestions that infection by certain viruses may predispose a person to MS. Certainly some viruses can cause demyelinating diseases, and it is tempting to speculate that virus infection plays a significant role in MS, but at present there is no definitive data implicating a particular virus.

Rheumatoid Arthritis Attacks Joints

Rheumatoid arthritis is a common autoimmune disorder, most often affecting women from 40 to 60 years old. The major symptom is chronic inflammation of the joints, although the hematologic, cardiovascular, and respiratory systems are also frequently affected. Many individuals with rheumatoid arthritis produce a group of auto-antibodies called **rheumatoid factors** that are reactive with determinants in the Fc region of IgG. The classic rheumatoid factor is an IgM antibody with that reactivity. Such auto-antibodies bind to normal circulating IgG, forming IgM-IgG complexes that are deposited in the joints. These immune complexes can activate the complement cascade, resulting in a type III hypersensitive reaction, which leads to chronic inflammation of the joints.

Animal Models for Autoimmune Diseases

Animal models for autoimmune diseases have contributed valuable insights into the mechanism of autoimmunity, to

TABLE 20-2 Experimental animal models of autoimmune diseases

Animal model	Possible human disease counterpart	Inducing antigen	Disease transferred by T cells
SPONTANEOUS AUTOIMMUNE DISEASES			
Nonobese diabetic (NOD) mouse	Insulin-dependent diabetes mellitus (IDDM)	Unknown	Yes
(NZB × NZW) F_1 mouse	Systemic lupus erythematosus (SLE)	Unknown	Yes
Obese-strain chicken	Hashimoto's thyroiditis	Thyroglobulin	Yes
EXPERIMENTALLY INDUCED AUTOIMMUNE DISEASES*			
Experimental autoimmune myasthenia gravis (EAMG)	Myasthenia gravis	Acetylcholine receptor	Yes
Experimental autoimmune encephalomyelitis (EAE)	Multiple sclerosis (MS)	Myelin basic protein (MBP); proteolipid protien (PLP)	Yes
Autoimmune arthritis (AA)	Rheumatoid arthritis	*M. tuberculosis* (proteoglycans)	Yes
Experimental autoimmune thyroiditis (EAT)	Hashimoto's thyroiditis	Thyroglobulin	Yes

* These diseases can be induced by injecting appropriate animals with the indicated antigen in complete Freund's adjuvant. Except for autoimmune arthritis, the antigens used correspond to the self-antigens associated with the human-disease counterpart. Rheumatoid arthritis involves reaction to proteoglycans, which are self-antigens associated with connective tissue.

our understanding of autoimmunity in humans, and to potential treatments. Autoimmunity develops spontaneously in certain inbred strains of animals and can also be induced by certain experimental manipulations (Table 20-2).

Autoimmunity Can Develop Spontaneously in Animals

A number of autoimmune diseases that develop spontaneously in animals exhibit important clinical and pathologic similarities to certain autoimmune diseases in humans. Certain inbred mouse strains have been particularly valuable models for illuminating the immunologic defects involved in the development of autoimmunity.

New Zealand Black (NZB) mice and F_1 hybrids of NZB and New Zealand White (NZW) mice spontaneously develop autoimmune diseases that closely resemble systemic lupus erythematosus. NZB mice spontaneously develop autoimmune hemolytic anemia between 2 and 4 months of age, at which time various auto-antibodies can be detected, including antibodies to erythrocytes, nuclear proteins, DNA, and T lymphocytes. F_1 hybrid animals develop glomerulonephritis from immune-complex deposits in the kidney and die prematurely by 18 months. As in human SLE, the incidence of autoimmunity in the (NZB × NZW)F_1 hybrids is greater in females.

An accelerated and severe form of systemic autoimmune disease resembling systemic lupus erythematosus develops in a mouse strain called MRL/*lpr/lpr*. These mice are homozygous for a gene called *lpr*, which has been identified as a defective *fas* gene. The *fas*-gene product is a cell-surface protein belonging to the TNF family of cysteine-rich membrane receptors (see Figure 12-6d). When the normal Fas protein interacts with its ligand, it transduces a signal that leads to apoptotic death of the Fas-bearing cells. This mechanism may operate in destruction of target cells by some CTLs (see Figure 14-9). Fas is known also to be essential in the death of hyperactivated peripheral $CD4^+$ cells. Normally, when mature peripheral T cells become activated, they are induced to express both Fas antigen and Fas ligand, When Fas-bearing cells come into contact with a neighboring activated cell bearing Fas ligand, the Fas-bearing cell is induced to die. It is also possible that Fas ligand can engage Fas from the same cell, inducing a cellular suicide. In the absence of Fas, mature peripheral T cells do not die, and these activated cells continue to proliferate and produce cytokines that result in grossly enlarged lymph nodes and spleen. Defects in *fas* expression similar to that found in the *lpr* mouse are observed in humans, and these can have severe consequences. However there is no link between *fas* expression and SLE in humans, which suggests that the *lpr* mouse may not be a true model for SLE.

Another important animal model is the nonobese diabetic (NOD) mouse, which spontaneously develops a form of diabetes that resembles human insulin-dependent dia-

betes mellitus (IDDM). Like the human disease, the NOD mouse disease begins with lymphocytic infiltration into the islets of the pancreas. Also, as in IDDM, there is a strong association between certain MHC alleles and the development of diabetes in these mice. Experiments have shown that T cells from diabetic mice can transfer diabetes to nondiabetic recipients. For example, when the immune system of normal mice is destroyed by lethal doses of x-rays and then is reconstituted with an injection of bone-marrow cells from NOD mice, the reconstituted mice develop diabetes. Conversely, when the immune system of still healthy NOD mice is destroyed by x-irradiation and then reconstituted with normal bone-marrow cells, the NOD mice do not develop diabetes. Various studies have demonstrated a pivotal role for $CD4^+$ T cells in the NOD mouse, and recent evidence implicates the T_H1 subset in disease development.

Several other spontaneous autoimmune diseases have been discovered in animals that have served as models for similar human diseases. Among these are *Obese*-strain chickens, which develop both humoral and cell-mediated reactivity to thyroglobulin resembling that seen in Hashimoto's thyroiditis.

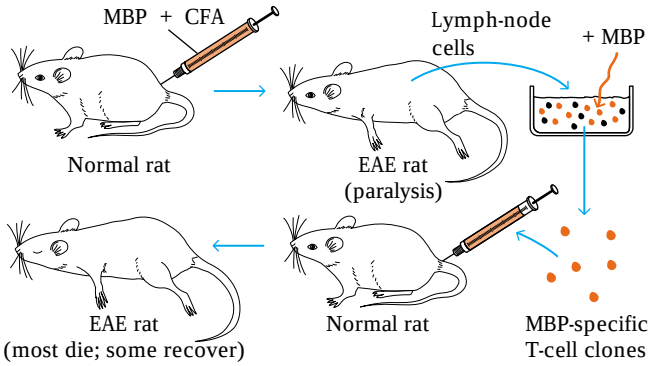

FIGURE 20-7 Experimental autoimmune encephalomyelitis (EAE) can be induced in rats by injecting them with myelin basic protein (MBP) in complete Freud's adjuvant (CFA). MBP-specific T-cell clones can be generated by culturing lymph-node cells from EAE rats with MBP. When these T cells are injected into normal animals, most develop EAE and die, although a few recover.

Autoimmunity Can Be Induced Experimentally in Animals

Autoimmune dysfunctions similar to certain human autoimmune diseases can be induced experimentally in some animals (see Table 20-2). One of the first such animal models was discovered serendipitously in 1973 when rabbits were immunized with acetylcholine receptors purified from electric eels. The animals soon developed muscular weakness similar to that seen in myasthenia gravis. This experimental autoimmune myasthenia gravis (EAMG) was shown to result when antibodies to the acetylcholine receptor blocked muscle stimulation by acetylcholine in the synapse. Within a year, this animal model had proved its value with the discovery that auto-antibodies to the acetylcholine receptor were the cause of myasthenia gravis in humans.

Experimental autoimmune encephalomyelitis (EAE) is another animal model that has greatly improved understanding of autoimmunity. This is one of the best-studied models of autoimmune disease. EAE is mediated solely by T cells and can be induced in a variety of species by immunization with myelin basic protein (MBP) or proteolipid protein (PLP) in complete Freund's adjuvant (Figure 20-7). Within 2–3 weeks the animals develop cellular infiltration of the myelin sheaths of the central nervous system, resulting in demyelination and paralysis. Most of the animals die, but others have milder symptoms, and some animals develop a chronic form of the disease that resembles chronic relapsing and remitting MS in humans. Those that recover are resistant to the development of disease from a subsequent injection of MBP and adjuvant.

The mouse EAE model provides a system for testing treatments for human MS. For example, because MBP- or PLP-specific T-cell clones are found in the periphery, it is assumed that these clones must have escaped negative selection in the thymus. Recent mouse experiments have suggested that orally administered MBP may make these antigen-specific peripheral T-cell clones self-tolerant. These studies have paved the way for clinical trials in MS patients.

Experimental autoimmune thyroiditis (EAT) can be induced in a number of animals by immunizing with thyroglobulin in complete Freund's adjuvant. Both humoral antibodies and T_H1 cells directed against the thyroglobulin develop, resulting in thyroid inflammation. EAT appears to best mimic Hashimoto's thyroiditis. In contrast to both EAE and EAT, which are induced by immunizing with self-antigens, autoimmune arthritis (AA) is induced by immunizing rats with *Mycobacterium tuberculosis* in complete Freund's adjuvant. These animals develop an arthritis whose features are similar to those of rheumatoid arthritis in humans.

Evidence Implicating the $CD4^+$ T Cell, MHC, and TCR in Autoimmunity

The inappropriate response to self-antigens that characterizes all autoimmune diseases can involve either the humoral or cell-mediated branches of the immune system. Identifying the defects underlying human autoimmune diseases has been difficult; more success has been achieved in characterizing the immune defects in the various animal models. Each of the animal models has implicated the $CD4^+$ T cell as the primary mediator of autoimmune disease. For example, the evidence is quite strong that, in mice, EAE is caused by $CD4^+$ T_H1 cells specific for the immunizing antigen. The disease can be transferred from one animal into another by T cells from animals immunized with either MBP or PLP or by

cloned T-cell lines from such animals. It also has been shown that disease can be prevented by treating animals with anti-CD4 antibodies. These data are compelling evidence for the involvement of CD4 in the establishment of EAE.

T-cell recognition of antigen, of course, involves a trimolecular complex of the T-cell receptor, an MHC molecule, and antigenic peptide (see Figure 9-16). Thus, an individual susceptible to autoimmunity must possess MHC molecules and T-cell receptors capable of binding self-antigens.

$CD4^+$ T Cells and T_H1/T_H2 Balance Plays an Important Role in Autoimmunity in Some Animal Models

Autoimmune T-cell clones have been obtained from all of the animal models listed in Table 20-2 by culturing lymphocytes from the autoimmune animals in the presence of various T-cell growth factors and by inducing proliferation of specific autoimmune clones with the various autoantigens. For example, when lymph-node cells from EAE rats are cultured in vitro with myelin basic protein (MBP), clones of activated T cells emerge. When sufficient numbers of these MBP-specific T-cell clones are injected intravenously into normal syngeneic animals, the cells cross the blood-brain barrier and induce demyelination; EAE develops very quickly, within 5 days (see Figure 20-7).

A similar experimental protocol has been used to isolate T-cell clones specific for thyroglobulin and for *M. tuberculosis* from EAT and AA animals, respectively. In each case, the T-cell clone induces the experimental autoimmune disease in normal animals. Examination of these T cells has revealed that they bear the CD4 membrane marker. In a number of animal models for autoimmune diseases it has been possible to reverse the autoimmunity by depleting the T-cell population with antibody directed against CD4. For example, weekly injections of anti-CD4 monoclonal antibody abolished the autoimmune symptoms in (NZB $\times$ NZW) F_1 mice and in mice with EAE.

Most cases of organ-specific autoimmune disease develop as a consequence of self-reactive $CD4^+$ T cells. Analysis of these cells has revealed that the T_H1/T_H2 balance can affect whether autoimmunity develops. T_H1 cells have been implicated in the development of autoimmunity, whereas, in a number of cases, T_H2 cells not only protect against the induction of disease but also against progression of established disease. In EAE, for example, immunohistologic studies revealed the presence of T_H1 cytokines (IL-2, TNF-α, and IFN-γ) in the central nervous system tissues at the height of the disease. In addition, the MBP-specific $CD4^+$ T-cell clones generated from animals with EAE, as shown in Figure 20-7, can be separated into T_H1 and T_H2 clones. Experiments have shown that only the T_H1 clones transfer EAE to normal healthy mice, whereas the T_H2 clones not only do not transfer EAE to normal healthy mice but also protect the mice against induction of EAE by subsequent immunization with MBP plus adjuvant.

Experiments that assessed the role of various cytokines or cytokine inhibitors on the development of EAE have provided further evidence for the different roles of T_H1 and T_H2 cells in autoimmunity. When mice were injected with IL-4 at the time of immunization with MBP plus adjuvant, the development of EAE was inhibited, whereas administration of IL-12 had the opposite effect, promoting the development of EAE. As noted in Chapter 12, IL-4 promotes development of T_H2 cells and IFN-γ, in addition to other cytokines such as IL-12, promotes development of T_H1 cells (see Figure 12-12). Thus, the observed effects of IL-4 and IL-12 on EAE development are consistent with a role for T_H1 cells in the genesis of autoimmunity.

Autoimmunity Can Be Associated with the MHC or with Particular T-Cell Receptors

Several types of studies have supported an association between expression of a particular MHC allele and susceptibility to autoimmunity, an issue covered in detail in Chapter 7. The strongest association between an HLA allele and an autoimmune disease is seen in ankylosing spondylitis, an inflammatory disease of vertebral joints. Individuals who have *HLA-B27* have a 90 times greater likelihood of developing ankylosing spondylitis than individuals with a different *HLA-B* allele. However, the existence of such an association should not be interpreted to imply that the expression of a particular MHC allele has caused the disease, because the relationship between MHC alleles and development of autoimmune disease is complex. It is interesting to note that, unlike many other autoimmune diseases, 90% of the cases of ankylosing spondylitis are male.

The presence of T-cell receptors containing particular V_α and V_β domains also has been linked to a number of autoimmune diseases, including experimental EAE and its human counterpart, multiple sclerosis. In one approach, T cells specific for various encephalitogenic peptides of MBP were cloned and their T-cell receptors analyzed. For example, T-cell clones were obtained from PL/J mice by culturing their T cells with the acetylated amino-terminal nonapeptide of MBP presented in association with a class II IA^u MHC molecule. Analysis of the T-cell receptors on these clones revealed a restricted repertoire of V_α and V_β domains: 100% of the T-cell clones expressed V_α 4.3, and 80% of the T-cell clones expressed V_β 8.2. In human autoimmune diseases, evidence for restricted TCR expression has been obtained for both multiple sclerosis and myasthenia gravis. The preferential expression of TCR variable-region genes in these autoimmune T-cell clones suggests that a single epitope might induce the clonal expansion of a small number of pathogenic T cells.

Proposed Mechanisms for Induction of Autoimmunity

A variety of mechanisms have been proposed to account for the T-cell–mediated generation of autoimmune diseases (Figure 20-8). Evidence exists for each of these mechanisms,

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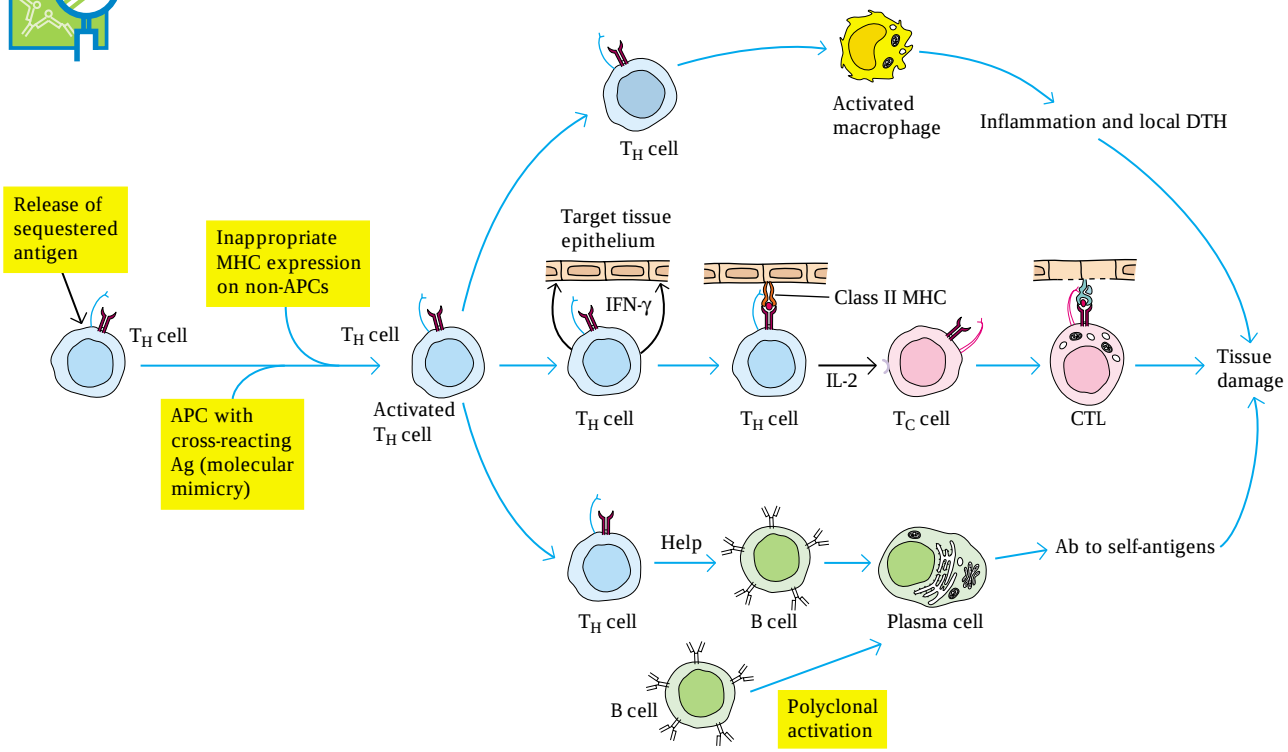

FIGURE 20-8 Proposed mechanisms for inducing autoimmune responses. Normal thymic selection appears to generate some self-reactive T_H cells; abnormalities in this process may generate even more self-reactive T_H cells. Activation of these self-reactive T cells in various ways, as well as polyclonal activation of B cells, is thought to induce an autoimmune response, in this case resulting in tissue damage. In all likelihood, several mechanisms are involved in each autoimmune disease. *[Adapted from V. Kumar et al., 1989,* Annu. Rev. Immunol. ***7:****657.]*

and it is likely that autoimmunity does not develop from a single event but rather from a number of different events.

In addition, susceptibility to many autoimmune diseases differs between the two sexes. As noted earlier, Hashimoto's thyroiditis, systemic lupus erythematosus, multiple sclerosis, rheumatoid arthritis, and scleroderma preferentially affect women. Factors that have been proposed to account for this preferential susceptibility, such as hormonal differences between the sexes and the potential effects of fetal cells in the maternal circulation during pregnancy, are discussed in the Clinical Focus.

Release of Sequestered Antigens Can Induce Autoimmune Disease

As discussed in Chapter 10, the induction of self-tolerance in T cells results from exposure of immature thymocytes to self-antigens and the subsequent clonal deletion of those that are self-reactive. Any tissue antigens that are sequestered from the circulation, and are therefore not seen by the developing T cells in the thymus, will not induce self-tolerance. Exposure of mature T cells to such normally sequestered antigens at a later time might result in their activation.

Myelin basic protein (MBP) is an example of an antigen normally sequestered from the immune system, in this case by the blood-brain barrier. In the EAE model, animals are injected directly with MBP, together with adjuvant, under conditions that maximize immune exposure. In this type of animal model, the immune system is exposed to sequestered self-antigens under nonphysiologic conditions; however, trauma to tissues following either an accident or a viral or bacterial infection might also release sequestered antigens into the circulation. A few tissue antigens are known to fall into this category. For example, sperm arise late in development and are sequestered from the circulation. However, after a vasectomy, some sperm antigens are released into the circulation and can induce auto-antibody formation in some men. Similarly, the release of lens protein after eye damage or of heart-muscle antigens after myocardial infarction has been shown to lead on occasion to the formation of auto-antibodies.

CLINICAL FOCUS

Why Are Women More Susceptible Than Men to Autoimmunity? Gender Differences in Autoimmune Disease

Of the nearly 9 million individuals in the United States with autoimmune disease, approximately 6.7 million are women. This predisposition to autoimmunity is more apparent in some diseases than others. For example, the female:male ratio of individuals who suffer from diseases such as multiple sclerosis (MS) or rheumatoid arthritis (RA) is approximately two or three females to one male, and there are nine women for every one male afflicted with systemic lupus erythematosus (SLE). However, these statistics do not tell the entire story, since, in some diseases, MS for example, the severity of the disease can be worse in men than in women. The fact that women are more susceptible to autoimmune disease has been recognized for several years but the reasons for this increased risk are not entirely understood. Here some of the possible explanations are considered.

Although it may seem unlikely, considerable evidence suggests there are significant gender differences in immune responses in both humans and mice. Immunization studies in both species suggest that females produce a higher titer of antibodies than males. In fact, females in general tend to mount more vigorous immune responses. In humans, this is particularly apparent in young females. Women tend to have higher levels of $CD4^+$ T cells and significantly higher levels of serum IgM.

In mice, whose gender differences are easier to study, there is a large body of literature documenting gender differences in immune responses. Female mice are much more likely than male mice to develop T_H1 responses and, in infections to which pro-inflammatory T_H1 responses are beneficial, are more likely to be resistant to the infection. An excellent example is infection by viruses such as vesicular stomatitis virus (VSV), herpes simplex virus (HSV), and Theiler's murine encephalomyelitis virus (TMEV). Clearance of these viruses is enhanced by T_H1 responses. In some cases, however, a pro-inflammatory response can be deleterious. For example, a T_H1 response to lymphocytic choriomeningitis virus (LCMV) correlates with more severe disease and significant pathology. Thus, female mice are more likely to succumb to infection with LCMV. The fact that gender is important in LCMV infection is underscored by experiments demonstrating that castrated male mice behave like females and are more likely to succumb to infection than their un-castrated male littermates.

Another disease in which gender plays a role is infection by coxsackie virus Type B-3 (CVB-3), an etiological agent of immune myocarditis. Male mice are much more susceptible to this disease than females. CVB-3 induces a predominant T_H1 response in males, while females, contrary to the situations described above, respond by mounting a protective T_H2 response. The response by females can be altered by injecting them with testosterone, which makes them susceptible to the disease. Additionally, the male response can be altered by injecting them with estradiol, which makes them resistant to the virus. These data in mice are consistent with the possibility that basic differences may well exist between men and women in their responses to pathogens. We must stress, however, that the particular gender differences observed in mice may not extend to human populations.

How do these gender differences arise? The evidence cited above that estradiol or testosterone can alter the outcome of infection by CVB-3 suggests a critical role for sex hormones. In humans it appears that estrogen on its own does not play a significant role in the etiology of either RA or MS, but there are indications that it may be important in SLE. This is suggested by data indicating that estrogen can stimulate autoantibody production in SLE-prone mice and these effects can be modulated by an anti-estrogenic compound. Such data imply that, at least in mice, estrogen is capable of triggering SLE-like autoimmunity. Additionally, androgens such as testosterone clearly play an important role in some autoimmune diseases. Female NOD mice are much more susceptible to spontaneous diabetes, and castration significantly increases the susceptibility of male NOD mice. Female SJL mice are more likely to be susceptible to EAE, a mouse MS-like disease. This indicates that testosterone may well be effective in ameliorating some autoimmune responses and so

Data indicate that injection of normally sequestered antigens directly into the thymus can reverse the development of tissue-specific autoimmune disease in animal models. For instance, intrathymic injection of pancreatic islet beta cells prevented development of autoimmunity in NOD mice. Moreover, EAE was prevented in susceptible rats by prior injection of MBP directly into the thymus. In these experiments, exposure of immature T cells to self-antigens that normally are not present in the thymus presumably led to tolerance to these antigens.

may be protective against several autoimmune diseases, including MS, diabetes, SLE, and Sjogren's syndrome.

Why do sex steroids affect immune responses? This is not well understood, but it is likely that these hormones, which circulate throughout the body, alter immune responses by altering patterns of gene expression. The sex steroids, a highly lipophilic group of compounds, function by passing through the cell membrane and binding a cytoplasmic receptor. Each hormone has a cognate receptor and binding of hormone to receptor leads to the activation or, in some instances, repression of gene expression. This is mediated by the binding of the receptor/hormone complex receptor to a specific DNA sequence. Thus, estrogen enters a cell, binds to the estrogen receptor, and induces the binding of the estrogen receptor to a specific DNA sequence, which in turn results in the modulation of transcription. Therefore, in cells that contain hormone receptors, sex hormones can regulate gene expression, and it is highly likely that sex steroids play an important role in the immune system through their receptors. Whether various cells of the immune system contain hormone receptors is not known at present; to understand how sex hormones mediate immune responses, clearly we must determine which cells express which hormone receptors.

Hormonal effects on immune responses may not be limited to steroidal sex hormones. Prolactin, a hormone that is expressed in higher levels in women than in men, is not a member of the lipophilic sex steroid family that includes estrogen, progesterone, and testosterone. But prolactin secretion (by the anterior pituitary) is stimulated by estrogen, thus explaining the higher levels of prolactin in women and the very high levels observed during pregnancy. Prolactin can have a profound influence on immune responses, as demonstrated in mice by removal of the anterior pituitary: this results in a severe immunosuppression, which can be entirely reversed by treatment with exogenous prolactin. The presence of prolactin receptors on peripheral T and B cells in humans is further evidence that this hormone may play a role in regulating immune responses. In fact, some evidence suggests that prolactin may tend to turn cells towards T_H1-dominated immune responses.

Pregnancy may give us a clue to how sex plays a role in regulating immune response. It is clear that, while women normally mount a normal response to foreign antigens, during pregnancy it is critical that the mother tolerate the fetus (which is, in fact, a foreign graft). This makes it very likely that the female immune system undergoes important modifications during pregnancy. Recall that women normally tend to mount more T_H1-like responses than T_H2 responses. During pregnancy, however, women mount more T_H2-like responses. It is thought that pregnancy-associated levels of sex steroids may promote an anti-inflammatory environment. In this regard, it is notable that diseases enhanced by T_H2-like responses, such as SLE, which has a strong antibody-mediated component, can be exacerbated during pregnancy, while diseases that involve inflammatory responses, such as RA and MS, sometimes are ameliorated in pregnant women.

Another effect of pregnancy is the presence of fetal cells in the maternal circulation (see the description of scleroderma on page 000). It is known that fetal cells can persist in the maternal circulation for decades, so these long-lived fetal cells may play a significant role in the development of autoimmune disease. Furthermore, the exchange of cells during pregnancy is bi-directional (cells of the mother may also appear in the fetal circulatory system), and this has led some to postulate that the presence of mother's cells in the male circulation could be a contributing factor in autoimmune disease.

In summary, women and men differ significantly in their ability to mount an immune response. Women mount more robust immune responses, and these responses tend to be more T_H1-like. It has been reported that estrogen is immunostimulatory; this may be due, in part, to the ability of the hormone to regulate specific gene expression through the estrogen receptor. Furthermore, the incidence of autoimmune diseases is sharply higher in women than in men. These observations have generated the compelling hypothesis that the tendency of females to mount more T_H1-like responses may, in part, explain differences in susceptibility to autoimmunity. Since this type of response is pro-inflammatory, it may enhance the development of autoimmunity. Whether the bias towards a T_H1 response is due to differences in sex steroids between males and females is less certain, but surely, in the next several years, experiments that explore this idea are likely to be pursued vigorously.

NOTE: The data discussed in this Clinical Focus were extracted from a letter to *Science* (C. C. Whitacre, S. C. Reingold, and P. A. O'Looney, 1999, *Science* **283:**1277) from the Task Force on Gender, MS, and Autoimmunity, a group convened by the National Multiple Sclerosis Society to begin a dialog on issues of gender and autoimmune disease. *Science* also has established a Web site (http://www.sciencemag.org/feature/data/983519.shl) that contains more detailed data concerning autoimmunity and gender.

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Molecular Mimicry May Contribute to Autoimmune Disease

For several reasons, the notion that microbial or viral agents might play a role in autoimmunity is very attractive. It is well accepted that migrant human populations acquire the diseases of the area to which they move and that the incidence of autoimmunity has increased dramatically as populations have become more mobile. This, coupled with the fact that a number of viruses and bacteria have been shown to possess

TABLE 20-3 Molecular mimicry between proteins of infectious organisms and human host proteins

Protein*	Residue†	Sequence‡
Human cytomegalovirus IE2	79	P D P L G R P D E D
HLA-DR molecule	60	V T E L G R P D A E
Poliovirus VP2	70	S T T K E S R G T T
Acetylcholine receptor	176	T V I K E S R G T K
Papilloma virus E2	76	S L H L E S L K D S
Insulin receptor	66	V Y G L E S L K D L
Rabies virus glycoprotein	147	T K E S L V I I S
Insulin receptor	764	N K E S L V I S E
Klebsiella pneumoniae nitrogenase	186	S R Q T D R E D E
HLA-B27 molecule	70	K A Q T D R E D L
Adenovirus 12 E1B	384	L R R G M F R P S Q C N
α-Gliadin	206	L G Q G S F R P S Q Q N
Human immunodeficiency virus p24	160	G V E T T T P S
Human IgG constant region	466	G V E T T T P S
Measles virus P3	13	L E C I R A L K
Corticotropin	18	L E C I R A C K
Measles virus P3	31	E I S D N L G Q E
Myelin basic protein	61	E I S F K L G Q E

*In each pair, the human protein is listed second. The proteins in each pair have been shown to exhibit immunologic cross-reactivity.

†Each number indicates the position on the intact protein of the amino-terminal amino acid in the listed sequence.

‡Amino acid residues are indicated by single-letter code. Identical residues are shown in blue.

SOURCE: Adapted from M. B. A. Oldstone, 1987, *Cell* **50**:819.

antigenic determinants that are identical or similar to normal host-cell components led Michael Oldstone to propose that a pathogen may express a region of protein that resembles a particular self-component in conformation or primary sequence. Such molecular mimicry appears in a wide variety of organisms (Table 20-3). In one study, 600 different monoclonal antibodies specific for 11 different viruses were tested to evaluate their reactivity with normal tissue antigens. More than 3% of the virus-specific antibodies tested also bound to normal tissue, suggesting that molecular mimicry is a fairly common phenomenon.

Molecular mimicry has been suggested as one mechanism that leads to autoimmunity. One of the best examples of this type of autoimmune reaction is post-rabies encephalitis, which used to develop in some individuals who had received the rabies vaccine. In the past, the rabies virus was grown in rabbit brain-cell cultures, and preparations of the vaccine included antigens derived from the rabbit brain cells. In a vaccinated person, these rabbit brain-cell antigens could induce formation of antibodies and activated T cells, which could cross-react with the recipient's own brain cells, leading to encephalitis. Cross-reacting antibodies are also thought to be the cause of heart damage in rheumatic fever, which can sometimes develop after a *Streptococcus* infection. In this case, the antibodies are to streptococcal antigens, but they cross-react with the heart muscle.

There Is Evidence for Mimicry Between MBP and Viral Peptides

Since the encephalitogenic MBP peptides are known, the extent to which they are mimicked by proteins from other organisms can be assessed. For example, one MBP peptide (amino acid residues 61–69) is highly homologous with a peptide in the P3 protein of the measles virus (see Table 20-3). In one study, the sequence of another encephalitogenic MBP peptide (66–75) was compared with the known sequences of a large number of viral proteins. This computer analysis revealed sequence homologies between this MBP peptide and a number of peptides from animal viruses, including influenza, polyoma, adenovirus, Rous sarcoma, Abelson leukemia, poliomyelitis, Epstein-Barr, and hepatitis B viruses.

One peptide from the polymerase enzyme of the hepatitis B virus was particularly striking, exhibiting 60% homology with a sequence in the encephalitogenic MBP peptide. To test the hypothesis that molecular mimicry can generate autoimmunity, rabbits were immunized with this hepatitis B virus peptide. The peptide was shown to induce both the formation of antibody and the proliferation of T cells that cross-reacted with MBP; in addition, central nervous system tissue from the immunized rabbits showed cellular infiltration characteristic of EAE.

These findings suggest that infection with certain viruses expressing epitopes that mimic sequestered self-components, such as myelin basic protein, may induce autoimmunity to those components. Susceptibility to this type of autoimmunity may also be influenced by the MHC haplotype of the individual, since certain class I and class II MHC molecules may be more effective than others in presenting the homologous peptide for T-cell activation.

Another particularly compelling example of molecular mimicry comes from studies of herpes stromal keratinitis (HSK). In these studies, investigators showed that prior infection of mice with herpes simplex virus Type 1 leads to a disease known as herpes stromal keratinitis (HSK), an autoimmune-like disease in which T cells specific for a particular viral peptide attack corneal tissue, thus causing blindness. These data demonstrated very clearly that a particular epitope of HSV-1 is responsible for the disease and that mutant strains of HSV-1 that lack this epitope do not cause HSK. The data provide strong evidence for molecular mimicry in the development of a particular autoimmune disease.

Inappropriate Expression of Class II MHC Molecules Can Sensitize Autoreactive T Cells

The pancreatic beta cells of individuals with insulin-dependent diabetes mellitus (IDDM) express high levels of both class I and class II MHC molecules, whereas healthy beta cells express lower levels of class I and do not express class II at all. Similarly, thyroid acinar cells from those with Graves' disease have been shown to express class II MHC molecules on their membranes. This inappropriate expression of class II MHC molecules, which are normally expressed only on antigen-presenting cells, may serve to sensitize T_H cells to peptides derived from the beta cells or thyroid cells, allowing activation of B cells or T_C cells or sensitization of T_H1 cells against self-antigens.

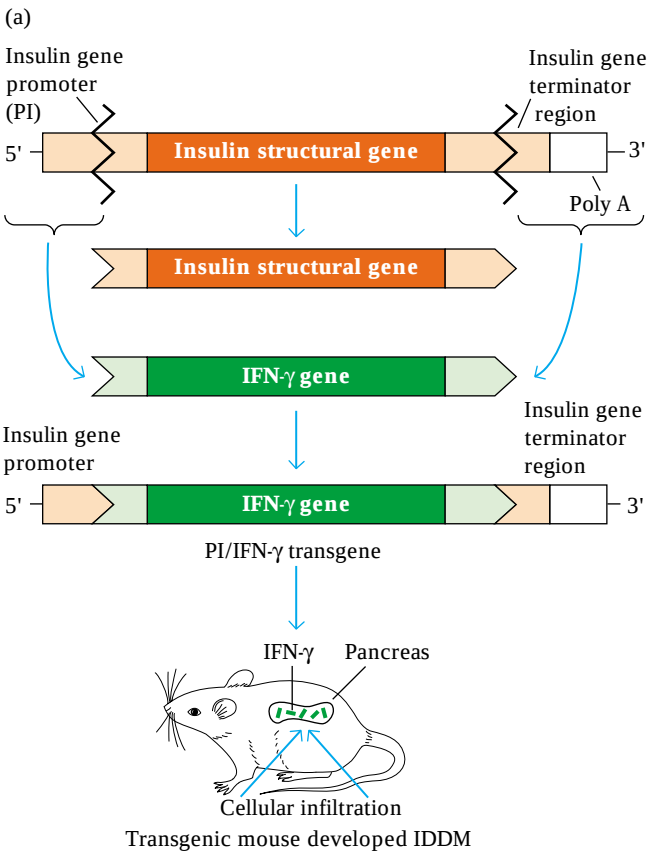
Other evidence suggests that certain agents can induce some cells that should not express class II MHC molecules to express them. For example, the T-cell mitogen phytohemagglutinin (PHA) has been shown to induce thyroid cells to express class II molecules. In vitro studies reveal that IFN-γ also induces increases in class II MHC molecules on a wide variety of cells, including pancreatic beta cells, intestinal epithelial cells, melanoma cells, and thyroid acinar cells. It was hypothesized that trauma or viral infection in an organ may induce a localized inflammatory response and thus increased concentrations of IFN-γ in the affected organ. If IFN-γ induces class II MHC expression on non-antigen-presenting cells, inappropriate T_H-cell activation might follow, with autoimmune consequences. It is noteworthy that SLE patients with active disease have higher serum titers of IFN-γ than patients with inactive disease. These data suggested that the increase in IFN-γ in these patients may lead to inappropriate expression of class II MHC molecules and thus to T-cell activation against a variety of autoantigens.

An interesting transgenic mouse system implicates IFN-γ and inappropriate class II MHC expression in autoimmunity. In this system, an IFN-γ transgene was genetically engineered with the insulin promoter, so that the transgenic mice secreted IFN-γ from their pancreatic beta cells (Figure 20-9a). Since IFN-γ up-regulates class II MHC expression, these transgenic mice also expressed class II MHC molecules on their pancreatic beta cells. The mice developed diabetes, which was associated with cellular infiltration of lymphocytes and inflammatory cells like the infiltration seen in autoimmune NOD mice and in patients with insulin-dependent diabetes mellitus (Figure 20-9b).

Although inappropriate class II MHC expression on pancreatic beta cells may be involved in the autoimmune reaction in these transgenic mice, other factors also may play a role. For example, IFN-γ is known to induce production of several other cytokines, including IL-1 and TNF. Therefore, the development of autoimmunity in this transgenic system may involve antigen presentation by class II MHC molecules on pancreatic beta cells together with a co-stimulatory signal, such as IL-1, that may activate self-reactive T cells. There is also some evidence to suggest that IL-1, IFN-γ, and TNF may directly impair the secretory function of human beta cells.

Polyclonal B-Cell Activation Can Lead to Autoimmune Disease

A number of viruses and bacteria can induce nonspecific polyclonal B-cell activation. Gram-negative bacteria, cytomegalovirus, and Epstein-Barr virus (EBV) are all known to be such polyclonal activators, inducing the proliferation of numerous clones of B cells that express IgM in the absence of T_H cells. If B cells reactive to self-antigens are activated by this mechanism, auto-antibodies can appear. For instance, during infectious mononucleosis, which is caused by EBV, a variety of auto-antibodies are produced, including auto-antibodies reactive to T and B cells, rheumatoid factors, and antinuclear antibodies. Similarly, lymphocytes from patients with SLE produce large quantities of IgM in culture, suggesting that they have been polyclonally activated. Many AIDS patients also show high levels of nonspecific antibody and auto-antibodies to RBCs and platelets. These patients are often coinfected with other viruses such as EBV and cytomegalovirus, which may induce the polyclonal B-cell activation that results in auto-antibody production.



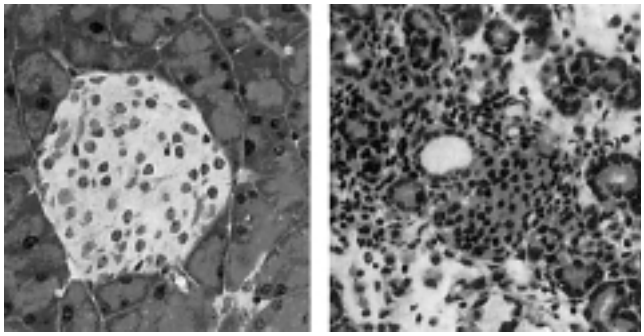


FIGURE 20-9 Insulin-dependent diabetes mellitus (IDDM) in transgenic mice. (a) Production of transgenic mice contalning an IFN-γ transgene linked to the insulin promoter (PI). The transgenics, which expressed the PI/IFN-γ transgene only in the pancreas, developed symptoms characteristic of IDDM. (b) Pancreatic islets of Langerhans from a normal BALB/c mouse (*left*) and from PI/IFN-γ transgenics at 3 weeks (*right*) showing infiltration of inflammatory cells. *[Part (b) from N. Sarvetnick, 1988,* Cell ***52:****773.]*

Treatment of Autoimmune Diseases

Ideally, treatment for autoimmune diseases should be aimed at reducing only the autoimmune response while leaving the rest of the immune system intact. To date, this ideal has not been reached.

Current therapies for autoimmune diseases are not cures but merely palliatives, aimed at reducing symptoms to provide the patient with an acceptable quality of life. For the most part, these treatments provide nonspecific suppression of the immune system and thus do not distinguish between a pathologic autoimmune response and a protective immune response. Immunosuppressive drugs (e.g., corticosteroids, azathioprine, and cyclophosphamide) are often given with the intent of slowing proliferation of lymphocytes. By depressing the immune response in general, such drugs can reduce the severity of autoimmune symptoms. The general reduction in immune responsiveness, however, puts the patient at greater risk for infection or the development of cancer. A somewhat more selective approach employs **cyclosporin A** or FK506 to treat autoimmunity. These agents block signal transduction mediated by the T-cell receptor; thus, they inhibit only antigen-activated T cells while sparing nonactivated ones.

Another therapeutic approach that has produced positive results in some cases of myasthenia gravis is removal of the thymus. Because patients with this disease often have thymic abnormalities (e.g., thymic hyperplasia or thymomas), adult thymectomy often increases the likelihood of remission of symptoms. Patients with Graves' disease, myasthenia gravis, rheumatoid arthritis, or systemic lupus erythematosus may experience short-term benefit from plasmapheresis. In this process, plasma is removed from a patient's blood by continuous-flow centrifugation. The blood cells are then resuspended in a suitable medium and returned to the patient. Plasmapheresis has been beneficial to patients with autoimmune diseases involving antigen-antibody complexes, which are removed with the plasma. Removal of the complexes, although only temporary, can result in a short-term reduction in symptoms.

On the positive side, studies with experimental autoimmune animal models have provided evidence that it is indeed possible to induce specific immunity to the development of autoimmunity. Several of these approaches are described below and outlined in Figure 20-10.

T-Cell Vaccination Is a Possible Therapy

The basis for T-cell vaccination as a therapy for some autoimmune diseases came from experiments with the EAE animal model. When rats were injected with low doses ($<10^{-4}$) of cloned T cells specific for MBP, they did not develop

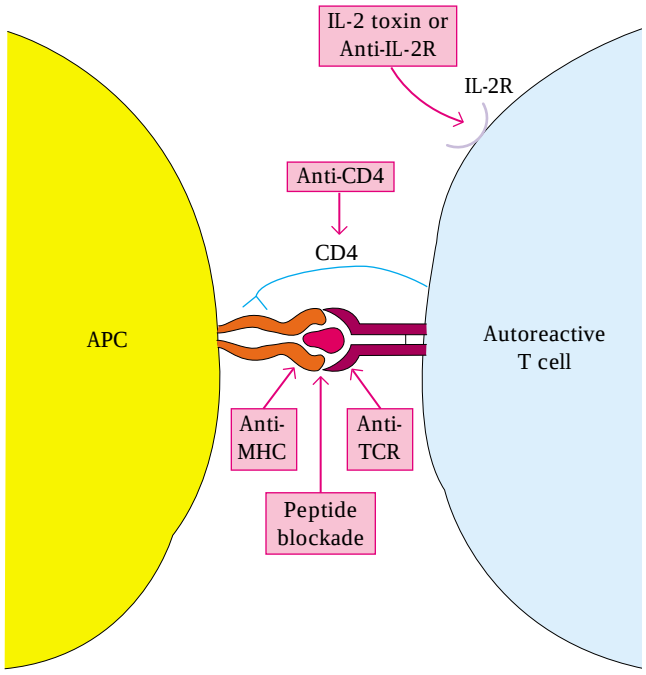


FIGURE 20-10 Some experimental agents for immunointervention in autoimmune disease.

symptoms of EAE. Instead they became resistant to the development of EAE when later challenged with a lethal dose of activated MBP-specific T cells or MBP in adjuvant. Later findings revealed that the efficacy of these autoimmune T-cell clones as a vaccine could be enhanced by crosslinking the cell-membrane components with formaldehyde or glutaraldehyde. When crosslinked T cells were injected into animals with active EAE, permanent remission of symptoms was observed. The crosslinked T cells apparently elicit regulatory T cells specific for TCR variable-region determinants of the autoimmune clones. Presumably these regulatory T cells act to suppress the autoimmune T cells that mediate EAE.

Peptide Blockade of MHC Molecules Can Modulate Autoimmune Responses

Identification and sequencing of various autoantigens has led to the development of new approaches to modulate autoimmune T-cell activity. In EAE, for example, the encephalitogenic peptides of MBP have been well characterized. Synthetic peptides differing by only one amino acid from their MBP counterpart have been shown to bind to the appropriate MHC molecule. Moreover, when sufficient amounts of such a peptide were administered along with the corresponding encephalitogenic MBP peptide, the clinical development of EAE was blocked. Presumably, the synthetic peptide acts as a competitor, occupying the antigen-binding cleft on MHC molecules and thus preventing binding of the MBP peptide.

In other studies, blocking peptides complexed to soluble class II MHC molecules reversed the clinical progression of EAE in mice, presumably by inducing a state of clonal anergy in the autoimmune T cells.

Monoclonal Antibodies May Be Used to Treat Autoimmunity

Monoclonal antibodies have been used successfully to treat autoimmune disease in several animal models. For example, a high percentage of (NZB × NZW) F_1 mice given weekly injections of high doses of monoclonal antibody specific for the CD4 membrane molecule recovered from their autoimmune lupus-like symptoms (Figure 20-11). Similar positive results were observed in NOD mice, in which treatment with an anti-CD4 monoclonal antibody led to disappearance of the lymphocytic infiltration and diabetic symptoms.

Because anti-CD4 monoclonal antibodies block or deplete all T_H cells, regardless of their specificity, they can threaten the overall immune responsiveness of the recipient. One remedy for this disadvantage is to try to block antigen-activated T_H cells only, since these cells are involved in the autoimmune state. To do this, researchers have used monoclonal antibody directed against the α subunit of the high-affinity IL-2 receptor, which is expressed only by antigen-activated T_H cells. Because the IL-2R α subunit is expressed at higher levels on autoimmune T cells, monoclonal antibody to the α subunit (anti-TAC) might preferentially block autoreactive T cells. This approach was tested in adult rats injected with activated MBP-specific T cells in the presence or absence of anti-TAC. All the control rats died of EAE, whereas six of the nine treated with anti-TAC had no symptoms, and the symptoms in the other three were mild.

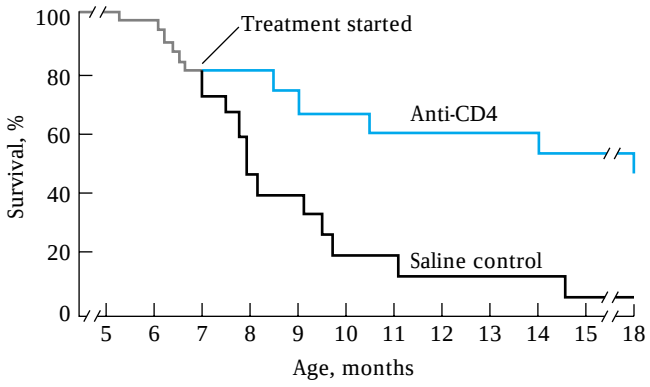


FIGURE 20-11 Weekly injections of anti-CD4 monoclonal antibody into (NZB × NZW) F_1 mice exhibiting autoimmune lupus-like symptoms significantly increased their survival rate. *[Adapted from D. Wofsy, 1988,* Prog. Allergy ***45:****106.]*

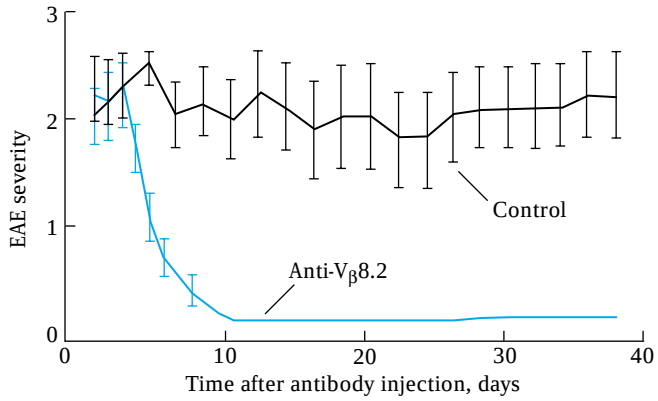


FIGURE 20-12 Injection of monoclonal antibody to the V_β 8.2 T-cell receptor into PL/J mice exhibiting EAE symptoms produced nearly complete remission of symptoms. EAE was induced by injecting mice with MBP-specific T-cell clones. EAE severity scale: 3 = total paralysis of lower limbs; 2 = partial paralysis of lower limbs; 1 = limb tail; 0 = normal (no symptoms). *[Adapted from H. Acha-Orbea et al., 1989,* Annu. Rev. Immunol. ***7:****371.]*

The association of autoimmune disease with restricted TCR expression in a number of animal models has prompted researchers to see if blockage of the preferred receptors with monoclonal antibody might be therapeutic. Injection of PL/J mice with monoclonal antibody specific for the V_β 8.2 T-cell receptor prevented induction of EAE by MBP in adjuvant. Even more promising was the finding that the V_β 8.2 monoclonal antibody could also reverse the symptoms of autoimmunity in mice manifesting induced EAE (Figure 20-12) and that these mice manifested long-term remission. Clearly, the use of monoclonal antibodies as a treatment for human autoimmune diseases presents exciting possibilities.

Similarly, the association of various MHC alleles with autoimmunity (see Table 7-4), as well as the evidence for increased or inappropriate MHC expression in some autoimmune disease, offers the possibility that monoclonal antibodies against appropriate MHC molecules might retard development of autoimmunity. Moreover, since antigen-presenting cells express many different class II MHC molecules, it should theoretically be possible to selectively block an MHC molecule that is associated with autoimmunity while sparing the others. In one study, injecting mice with monoclonal antibodies to class II MHC molecules before injecting MBP blocked the development of EAE. If, instead, the antibody was given after the injection of MBP, development of EAE was delayed but not prevented. In nonhuman primates, monoclonal antibodies to HLA-DR and HLA-DQ have been shown to reverse EAE.

Oral Antigens Can Induce Tolerance

When antigens are administered orally, they tend to induce the state of immunologic unresponsiveness called **tolerance.** For example, as mentioned earlier in this chapter, mice fed MBP do not develop EAE after subsequent injection of MBP. This finding led to a double-blind pilot trial in which 30 individuals with multiple sclerosis were fed either a placebo or 300 mg of bovine myelin every day for a year. The results of this study revealed that T cells specific for MBP were reduced in the myelin-fed group; there also was some suggestion that MS symptoms were reduced in the male recipients (although the reduction fell short of statistical significance) but not in the female recipients. While the results of oral tolerance induction in mice were promising, the data from humans do not appear to be as beneficial. However, the human clinical trials are in the early stages, and it may be that the peptides used so far were not the most effective, or perhaps the doses were not correct. Because of the promise of this approach as shown in animal studies, it is likely that more clinical trials will be conducted over the next few years.

SUMMARY

- Human autoimmune diseases can be divided into organ-specific and systemic diseases. The organ-specific diseases involve an autoimmune response directed primarily against a single organ or gland. The systemic diseases are directed against a broad spectrum of tissues and have manifestations in a variety of organs resulting from cell-mediated responses and cellular damage caused by auto-antibodies or immune complexes.
- There are both spontaneous and experimental animal models for autoimmune diseases. Spontaneous autoimmune diseases resembling systemic lupus erythematosus occur in NZB and (NZB × NZW) F_1 mice and in MRL/*lpr/lpr* mice, which have a defective *fas* gene. Several experimental animal models have been developed by immunizing animals with self-antigens in the presence of adjuvant.
- Studies with experimental autoimmune animal models have revealed a central role for $CD4^+$ T_H cells in the development of autoimmunity. In each of the experimentally induced autoimmune diseases, autoimmune T-cell clones can be isolated that induce the autoimmune disease in normal animals. The relative number of T_H1 and T_H2 cells appears to play a pivotal role in determining whether autoimmunity develops: T_H1 cells promote the development of autoimmunity, whereas T_H2 cells appear to block development of autoimmune disease and also block the progression of the disease once it is established. The MHC haplotype of the experimental animal determines the ability to present various autoantigens to T_H cells.
- A variety of mechanisms have been proposed for induction of autoimmunity, including release of sequestered antigens, molecular mimicry, inappropriate class II MHC expression on cells (in some cases stimulated by IFN-γ, and polyclonal B-cell activation. Evidence exists for each of these mecha-

nisms, reflecting the many different pathways leading to autoimmune reactions.

- Current therapies for autoimmune diseases include treatment with immunosuppressive drugs, thymectomy, and plasmapheresis for diseases involving immune complexes. Other therapies include vaccination with T cells specific for a given autoantigen, administration of synthetic blocking peptides that compete with autoantigen for binding to MHC molecules, treatment with monoclonal antibodies that react with some component specifically involved in an autoimmune reaction, and induction of tolerance to autoantigens by administering them orally.

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USEFUL WEB SITES

http://www.lupus.org/index.html

The site for the Lupus Foundation of America contains valuable information for patients and family members as well as current information about research in this area.

http://www.nih.gov/niams/

Home page for the National Institute for Arthritis and Musculoskeletal and Skin Diseases. This site contains links to other arthritis sites.

http://www.niddk.nih.gov/

Home page for the National Institute for Diabetes and Digestive and Kidney Diseases. This site contains an exhaustive list of links to other diabetes health-related sites.

http://www.sciencemag.org/feature/data/983519.shl

Link to a Web site that provides specific information concerning the role of gender in autoimmune disease.

Study Questions

CLINICAL FOCUS QUESTION What are some of the possible reasons why females are more susceptible to autoimmune diseases than males?

1. For each of the following autoimmune diseases (a–m), select the most appropriate characteristic (1–13) listed below.

Diseases

a. _____ Experimental autoimmune encephalitis (EAE)
b. _____ Goodpasture's syndrome
c. _____ Graves' disease
d. _____ Systemic lupus erythematosus (SLE)
d. _____ Insulin-dependent diabetes mellitus (IDDM)

Go to www.whfreeman.com/immunology Self-Test
Review and quiz of key terms

f. _____ Rheumatoid arthritis
g. _____ Hashimoto's thyroiditis
h. _____ Experimental autoimmune myasthenia gravis (EAMG)
i. _____ Myasthenia gravis
j. _____ Pernicious anemia
k. _____ Multiple sclerosis
l. _____ Autoimmune hemolytic anemia

Characteristics

(1) Auto-antibodies to intrinsic factor block vitamin B_{12} absorption
(2) Auto-antibodies to acetylcholine receptor
(3) T_H1-cell reaction to thyroid antigens
(4) Auto-antibodies to RBC antigens
(5) T-cell response to myelin
(6) Induced by injection of myelin basic protein plus complete Freund's adjuvant
(7) Auto-antibody to IgG
(8) Auto-antibodies to basement membrane
(9) Auto-antibodies to DNA and DNA-associated protein
(10) Auto-antibodies to receptor for thyroid-stimulating hormone
(11) Induced by injection of acetylcholine receptors
(12) T_H1-cell response to pancreatic beta cells

2. Experimental autoimmune encephalitis (EAE) has proved to be a useful animal model of autoimmune disorders.

a. Describe how this animal model is made.
b. What is unusual about the animals that recover from EAE?
c. How has this animal model indicated a role for T cells in the development of autoimmunity?

3. Molecular mimicry is one mechanism proposed to account for the development of autoimmunity. How has induction of EAE with myelin basic protein contributed to the understanding of molecular mimicry in autoimmune disease?

4. Describe at least three different mechanisms by which a localized viral infection might contribute to the development of an organ-specific autoimmune disease.

5. Transgenic mice expressing the IFN-γ transgene linked to the insulin promoter developed diabetes.

a. Why was the insulin promoter used?
b. What is the evidence that the diabetes in these mice is due to autoimmune damage?
c. What is unusual about MHC expression in this system?
d. How might this system mimic events that might be caused by a localized viral infection in the pancreas?

6. Monoclonal antibodies have been administered for therapy in various autoimmune animal models. Which monoclonal antibodies have been used and what is the rationale for these approaches?

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

a. T_H1 cells have been associated with development of autoimmunity.
b. Immunization of mice with IL-12 prevents induction of EAE by injection of myelin basic protein plus adjuvant.
c. The presence of the *HLA B27* allele is diagnostic for ankylosing spondylitis, an autoimmune disease affecting the vertebrae.
d. Individuals with pernicious anemia produce antibodies to intrinsic factor.
e. A defect in the gene encoding Fas can reduce programmed cell death by apoptosis.