Autoimmune Diseases in Small Animals

Laurel J. Gershwin, DVM, PhD

The function of the immune system is to protect the host from pathogens. The complex system of humoral and cellular immune components that interact to provide this protection depends on an ability to differentiate self from nonself. Early in fetal development, the thymus “educates” fetal thymic lymphocytes so that those that enter the periphery and become mature T lymphocytes do not react in an adverse way with host cells and tissues and are able to assist in the elimination of pathogens and other foreign cells that enter the host. Nonetheless, there are situations in which an immune response may be generated such that self-tissues are attacked. These responses are referred to as autoimmune and, depending on which of the self-antigens the immune response is directed toward, clinical signs of disease occur and are relevant to the functions of those target tissues or organs. For example, in autoimmune hemolytic anemia, antibodies bind specifically with antigenic epitopes on self-erythrocytes causing loss of red blood cells and subsequent anemia.

Thymic education of fetal thymocytes takes place in the thymic cortex where there are epithelial cells that express a wide variety of tissue antigens and major histocompatibility (MHC) antigens class I and II. The immature T cells are “tested” for their ability to bind to self-MHC antigens. Those that do not bind at all are subject to induction of apoptosis and are eliminated. Those that bind too strongly are similarly disposed of. The T cells with ability to recognize MHC of self but do not bind strongly enough to elicit a cytotoxic event are retained. These cells become CD4 or CD8 T cells and can bind to MHC class II or MHC class I, respectively, whereas their T-cell receptor (TCR) for antigen has specificity for some foreign epitope. The TCRs are screened for reactivity to the promiscuously expressed tissue antigens on thymic epithelial cells, and those that react with any of these antigens are induced into apoptosis and eliminated from the T-cell pool that enters the periphery to seed the secondary lymphoid organs.
Although the majority of B cells are tolerized to self-antigens, the specificity of B-cell receptors on B lymphocytes is not as rigorously controlled as that of T lymphocytes. There are B cells present in the body that are capable of recognizing and binding to some self-epitopes. The lack of T cells reactive with those antigens, however, keeps the B cells in check because they require T-cell help to initiate an immune response and antibody production.

There are several well-recognized pathogenic mechanisms for induction of autoimmune responses, and there are also many autoimmune diseases for which there is no known reason for development of the autoimmune response. One well-recognized mechanism occurs when the target tissue is in a privileged site, such that the T and B cells were never exposed to its tissue specific antigens during development. These sites include central nervous system tissues, the lens of the eye, and sperm-forming cells in the male testicle. If a traumatic event exposes these tissues to the adult immune system, an immune attack on the organ or tissue is a common sequel. Another well-recognized mechanism occurs when there are shared antigenic epitopes between a host tissue and a pathogen, such as a virus or bacteria. The presence of helper T cells specific for the pathogen makes it possible for B cells that are not tolerized to the cross-reactive antigens to use those T cells to establish the costimulatory signals required for activation and differentiation into antibody-producing plasma cells. The resultant antibodies can then attack the self-tissues and evoke inflammation and tissue destruction. Such is the case in rheumatic fever and heart disease in human patients infected with group A streptococci that cross-react with myocardial antigens.

A less well understood mechanism for development of autoimmune responses is the loss of suppression, which in current immunologic terms involves an alteration in regulatory T cell function. In the 1980s, the concept of T suppressor cells that held autoreactive lymphocytes in check was expanded to explain development of autoimmune responses.

Recently, the discovery of T helper 17 (Th17) cells and their role in chronic inflammatory and autoimmune disorders has enhanced understanding of important regulatory mechanisms. Human patients with Hashimoto thyroiditis, a T cell–mediated autoimmune disease that dogs and human patients develop, showed increased levels of T cells synthesizing IL-17 and IL-22 in peripheral blood when compared with controls. IL-17–secreting Th17 cells have been identified as active components in the pathogenesis of multiple sclerosis in human patients and immune-mediated experimental encephalitis in animal models. IL-17 is a proinflammatory cytokine and is implicated in the chronic autoimmune inflammation seen in rheumatoid arthritis (RA) patients.

Autoimmune disease was first recognized as rheumatic disease in the 1800s and was later referred to by Ehrlich as horror autotoxicus. Since those early descriptions, myriad autoimmune diseases have been recognized in humans. The recognition of autoimmune disease in domestic animals has lagged somewhat behind that for humans. Currently, autoimmune etiology is implicated in a variety of inflammatory diseases in dogs and cats, with representative disorders affecting most body systems. Although the pathogenesis of these diseases vary, all are caused by antibody or T-cell responses to self-antigens.

SYSTEMIC AUTOIMMUNE DISEASE: SYSTEMIC LUPUS ERYTHEMATOSUS

Initially recognized in human patients, systemic lupus erythematosus (SLE) is the autoimmune disease with the most diverse clinical presentation. The cause of SLE is unknown, but it is characterized by the production of primarily nonorgan-specific autoantibodies. These autoantibodies are directed against self-molecules found in
the cell nucleus. Autoantibodies to cell surface antigens are also found in this disease. In human patients there is a genetic predisposition for SLE (highest incidence is in African Americans) and a higher incidence rate in women. Several MHC class II genes have been implicated.

In dogs there is also a genetic predisposition; it is most commonly seen in collies, German shepherds, and Shetland sheepdogs. Other breeds, such as Irish setters and poodles, may be affected. Several MHC class I antigens (DLA-A7) are associated with an increased incidence of SLE. A canine patient presenting with SLE may show clinical signs relevant to the skin, kidney, joints, or hematologic system. The guidelines for diagnosis of SLE in human patients as established by the American College of Rheumatology include a positive antinuclear antibody (ANA) test or lupus erythematosus cell preparation and documented involvement of at least 2 body systems. In a 1993 study, the most common clinical signs were polyarthritis (in 91% of cases), renal involvement (65%), and mucocutaneous disorders (60%). Only 13% of patients had hemolytic anemia. ANAs were detected using indirect immunofluorescence assay (IFA) at titers of 256 and over. These titers correlated with the severity and the stage of the disease.

As discussed previously, diagnosis of SLE requires that patients have a positive ANA titer and the involvement of at least 2 body systems. In dogs with SLE, ANAs commonly recognize histones or soluble nuclear antigen whereas human ANA specificities favor double-stranded DNA as the antigen. The ANA test is usually performed by IFA using fixed HEP-2 cells derived from mouse liver tissue as antigen. The pattern of immunofluorescence and the titer can be detected and reported. The speckled and homogeneous patterns are most commonly recognized in canine sera. A recent study examined 120 dogs with and without positive ANA and determined that in those showing involvement of 1 or more body systems suggestive of systemic autoimmune disease, a positive ANA test was most likely to be predictive for SLE whereas a positive ANA test in the absence of at least 1 body system involved was not a good predictor for diagnosis of SLE. The presence of a decreased albumin/globulin ratio on blood analysis reflects the polyclonal activation of B cells with production of large amounts of immunoglobulin. Unlike the low albumin/globulin ratio in multiple myeloma, the densitometry tracing reveals a broad band reflecting the multiple clonality of the B cells that have been activated.

Pathogenic mechanisms in canine SLE involve development of immune complexes between antibodies specific for nuclear components and the liberated nuclear antigens in the circulation. When immune complexes deposit in kidney glomeruli, capillary networks in the joints, and the skin, a type III hypersensitivity reaction ensues and tissue damage results. Thus, fixation of complement liberates small fragments (C3a and C5a), which are chemotactic for neutrophils. The neutrophils release destructive enzymes in the tissues that contain immune complexes and inflammation and tissue necrosis occur. Resultant pathology gives rise to leaky glomerular capillaries and proteinuria, joint inflammation with resultant arthritis, and skin lesions. Some or all of these occur depending on the location of the immune reaction.

In SLE patients, other autoantibodies can occur, causing clinical signs relevant to different body systems. For example, if autoantibodies specific for erythrocyte antigens are made, hemolytic anemia may be part of the SLE complex. In this case, a Coombs test for antiererythrocyte antibodies is positive and the hematologic system counts for 1 affected body system. Similarly the presence of antithrombocyte antibodies causes thrombocytopenia. The pathogenesis of both of these conditions involves a type II hypersensitivity reaction in which antibodies bind the target cells and opsonize for removal by the fixed phagocyte system in the spleen or...
complement-mediated lysis. Thus, it is understandable that SLE patients may present with a shifting leg lameness due to arthritis, kidney failure due to immune complex-mediated glomerular nephritis, or anemic crisis due to Coombs-positive anemia or bleeding from thrombocytopenia.

Immune-mediated polyarthritis is a common component of SLE. Arthrocentesis from hock and carpal joints reveals an increase in neutrophilic inflammation in the absence of microorganisms. Joint taps are useful to follow the response of a patient to immunosuppressive therapy.

SLE patients presenting with erythematous lesions on the face will likely have immune complex deposition at the dermal epidermal junction (Fig. 1). Lesions on the nasal planum are common. Immunofluorescence staining of a biopsy from affected areas when stained with antisera specific for IgG or third component of complement (C3) shows a fluorescent band at the dermal epidermal junction. This lupus band is characteristic for SLE skin lesions (see Fig. 1B). There is a similar skin pathology that occurs in the absence of other body system involvement and in which patient serum is negative for ANAs; this condition is referred to as discoid lupus (DL). The lesions on the nasal planum are similar. DL and the skin manifestation of SLE are exacerbated by direct exposure to UV light.

Although the pathogenesis of SLE is well understood, the inciting cause for induction of the autoimmune response is usually elusive. Virus infection or exposure to environmental toxicants and other chemicals is often proposed. There are some definitive links to causal agents in the case of feline hyperthyroid patients treated with 6-propylthiouracil. These patients develop a lupus-like syndrome with formation of antibodies to native DNA. The development of this syndrome seems to be dose dependent.9

**Fig. 1.** (A) Erosive lesions on nasal planum in canine SLE. (B) Direct immunofluorescence on biopsy (from lesions in [A]) using antisera against canine IgG shows antibody binding at dermal epidermal junction, the lupus band.
ORGAN SYSTEM–SPECIFIC AUTOIMMUNE DISEASE

Pathogenic mechanisms for organ-specific autoimmune diseases most commonly involve the development of autoantibodies specific for 1 or more antigens on the target tissue. The destruction of the targeted cells occurs by a type II hypersensitivity mechanism in which antibodies bind the cells and cause lysis/membrane damage leading to cell death or removal by the fixed macrophages in liver and splenic sinusoids. In some instances, the autoimmune effector is sensitized T lymphocytes. In this case, the target organ is infiltrated with lymphocytes and other mononuclear cells. When T cells mediate damage, destruction of cells and tissue occurs by cell-mediated induction of apoptosis.

AUTOIMMUNE DISEASES OF THE HEMATOLOGIC SYSTEM

Immune-Mediated Hemolytic Anemia

In dogs, immune-mediated hemolytic anemia (IMHA) is a common cause of anemia, and in cats it is somewhat less common but not infrequent. Canine patients are often middle-aged female dogs, but in cats, males, at least in 1 study, were overrepresented. On presentation, IMHA patients show depression, pallor, and sometimes jaundice. The diagnosis of IMHA is suspected when a hemogram reveals spherocytosis and a regenerative anemia; this is usually accompanied by a positive Coombs test. IMHA can be idiopathic or instigated by 1 of several drugs, such as β-lactam antibiotics. In the latter case, metabolic products of the drug bind to erythrocytes creating a new epitope, which stimulates the production of antibodies that bind to the erythrocyte, fix complement, and initiate cell lysis or removal by fixed phagocytes in the spleen. It is likely, but not yet well documented, that overvaccination may serve as an inciting cause of IMHA in dogs. Polyclonal activation of B cells could induce autoantibody formation, particularly in genetically predisposed dogs.

A positive result from the direct Coombs test is a useful predictor of disease in dogs, but in cats, false-positive results are more frequent than in dogs. One study of IMHA in cats showed that the median packed cell volume on presentation was 12%. In more than 50% of the cats, the anemia was not regenerative. Additional abnormal laboratory results included leukocytosis, lymphocytosis, hyperbilirubinemia, and hyperglobulinemia.

The pathogenesis of IMHA varies depending on the isotype and specificity of the autoantibody produced. In IMHA, erythrocytes are targeted by antierythrocyte antibodies and anemia is the dominant clinical sign. When hemoglobinuria and hemoglobinemia are present, IgM is usually the predominant antibody because it causes complement-mediated lysis with subsequent icterus and hemoglobinemia. In contrast, an IgG antibody (so-called incomplete antibody) leads to anemia with low hematocrit and no hemolysis. This latter type of presentation is caused primarily by loss of erythrocytes from phagocytic destruction after being opsonized by IgG antibody. These cases generally show splenomegaly and sometimes also hepatomegaly.

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There are some dog breeds that have an increased incidence of IMHA. These include cocker spaniel, miniature schnauzer, beagle, Samoyed, and old English sheepdog. An association between a DLA class II haplotype and an increased incidence of IMHA has been described. In a study of 108 patients with Coombs-positive anemia, the 2 haplotypes that were increased relative to a breed-matched control cohort were: DLA-DRB1*00601/DQA1*005011/DQB1*00701, reported in dogs with warm reactive agglutinins; and DLA-DRB1*015/DQA1*00601/DQB1*00301 in dogs.
with both warm and cold reactive agglutinins. These results are similar to the associations found for human IMHA patients.

Treatment of IMTP, as of other diseases of autoimmune origin, requires vigorous immunosuppressive therapy, usually immunosuppressive doses of corticosteroids. Adjunct therapy may include the use of intravenous human immunoglobulin (to block Fc receptors on phagocytes), transfusion (only for animals in dire need of red blood cells), and other supportive care for animals in anemic crisis.\(^{11,15}\)

IMHA can occur by itself or with immune-mediated thrombocytopenia (IMTP), a condition known as Evans syndrome. The combination of erythrocyte loss and thrombocyte depletion creates a severe disease in which erythrocyte loss by immune depletion is supplemented by loss due to bleeding. In 1 study of 21 cases of IMHA and IMTP, there was overrepresentation of Airedale terriers and Dobermans. Less than 50% of the dogs survived for 30 days after original hospitalization, usually for bleeding disorders.\(^{16}\)

**Immune-Mediated Thrombocytopenia**

Primary idiopathic IMTP occurs without a known inciting cause and is more common in middle-aged female dogs. Cocker spaniels and old English sheepdogs are overrepresented. Patients with IMTP may present with prolonged bleeding, petechia, or ecchymosis. The presence of antibodies reactive with thrombocytes can be confirmed using the antimegakaryocyte antibody test on bone marrow or an indirect immunofluorescent evaluation of platelet-bound antibodies (PBAs) in the peripheral blood using flow cytometry. The detection of these antibodies is diagnostic for IMTP. In 1 recent study involving 83 thrombocytopenic dogs, 45% were found to have PBAs, as determined by flow cytometry.\(^{17}\) Increased megakaryopoiesis was observed in all dogs that were suspected of having ITP but in only 39% of dogs without PBAs.

Treatment of IMTP involves the use of immunosuppressive therapy (usually prednisone and cyclophosphamide to induce remission and azathioprine to maintain remission). The use of blood or packed cell transfusion depends on the hematocrit and need for blood. In a recent study, Horgan and colleagues\(^{18}\) found that splenectomy as an adjunct to immunosuppressive therapy was associated with improved outcome. Response to immunosuppressive therapy usually results in an increase of platelet levels to normal. Treatment of IMTP with intravenous human immunoglobulin has been evaluated by several groups. This procedure is often used in human patients with IMTP, as in IMHA, and is based on the principle that the human immunoglobulin blocks the Fc receptors on mononuclear cells in patients, thus precluding removal of opsonized platelets. In 1 small study, the use of this treatment seemed to result in rapid rise of platelet counts and was not associated with adverse side effects.\(^{19}\)

Idiopathic IMTP is most common; however, IMTP can occur secondary to drug therapy or infection. Several reported cases of ITP were linked to consumption of medications (anticonvulsants or antibiotics)—these cases are secondary ITP. In 1 study, 44 dogs infected naturally with *Leishmania infantum* were divided into those with and those without thrombocytopenia. Blood was tested for PBAs by IFA and 19 of 20 dogs with thrombocytopenia and 13 of 24 dogs without thrombocytopenia were positive by IFA. In contrast, 0 of 10 uninfected normal dogs were positive for PBAs.\(^{20}\)

**Immune-Mediated Neutropenia**

Loss of neutrophils by immune destruction is the least common of the immune-mediated hematologic diseases. The presence of antibodies reactive with neutrophils has been documented, however, in several human syndromes and in dogs. Neutropenia can occur alone or in conjunction with thrombocytopenia. In 1 case report, immune-mediated neutropenia and thrombocytopenia was described in 3 giant schnauzer dogs.\(^{21}\)
Two of these dogs had antineutrophil antibodies that were demonstrated by indirect agglutination (using Coombs reagent); in the third, IFA was used and it failed to detect antineutrophil antibodies. Patients with neutropenia present may present with recurrent bacterial infections due to the loss of a primary innate defense mechanism.

**AUTOIMMUNE DISEASES OF THE ENDOCRINE SYSTEM**

**Autoimmune Thyroiditis**

Autoimmune thyroiditis (AT), Hashimoto disease, is one of the most common autoimmune diseases in humans. It is characterized by infiltration of the thyroid gland with lymphocytes. Loss of thyroid function results in hypothyroidism. Development of antithyroxin antibodies is seen in 60% to 80% of these patients; in addition, antibodies to thyroperoxidase are present in up to 95% of patients and are considered to be superior as a predictive indicator of disease.

Hypothyroidism is a common disorder of dogs, with certain breeds showing enhanced predisposition. Primary hypothyroidism in which the thyroid is infiltrated by lymphocytes is considered an immune-mediated disease, with histologic similarities to Hashimoto thyroiditis in humans. Antibodies to circulating T3 or to T4 are often detectable. The development of clinical signs of lethargy, dermatologic changes, and obesity are not usually seen until at least 75% of the gland has been destroyed. Measurement of blood levels of T4 is below normal, and up to 80% of these patients have detectable autoantibodies to thyroglobulin. In dogs, hypothyroidism is most often manifested in middle age and is associated with obesity, mental dullness, alopecia primarily on the trunk, often secondary pruritic seborrhea sicca with or without otitis externa, hyperpigmentation, myxedema, and weakness. Other body systems can also be affected including the cardiovascular, gastrointestinal, and hematologic systems. Anemia is commonly associated with untreated AT.

There are many purebred dog breeds that have a higher than normal incidence of AT. Some of these include Doberman pinschers, golden retrievers, beagles, old English sheepdogs, Rhodesian ridgebacks, and many others. The disease is far less common in mixed breed dogs. Recent studies on MHC polymorphisms in affected dogs have identified several DLA antigens with increased representation in AT-affected dogs. Kennedy and colleagues have identified a significant association between DLA-DQA1*00101 with hypothyroidism. These investigators note that several breeds (Siberian husky, shih tzu, and Yorkshire terrier) that are not associated with AT have a low frequency of expression of DLA-DQA1*00101. Immunoendocrinopathy syndromes may occur in AT dogs, with patients developing first AT and then type 1 diabetes mellitus (DM) or hypoadrenocorticism. This is not surprising because there is evidence that the DLA-DQA1*001 allele is associated with DM and AT. In a recent study using giant schnauzers and Hovawart dogs, Ferm and colleagues examined birth cohorts for the presence of antithyroid autoantibodies (ATAs) and for elevated thyrotropin levels. Although both breeds had members with clinical hypothyroidism present, the number of dogs testing positive for ATAs and having high thyrotropin levels was greater than the number of dogs with clinical disease, indicating the potential prognostic value of ANA and thyrotropin level testing in breeds predisposed to AT.

Detection of ATAs in serum of hypothyroid dogs is a useful diagnostic aid. The role of these antibodies in pathogenesis, however, is not established. Studies performed by Choi and colleagues have demonstrated that there is a Th1 skew in AT patients. The destructive role of the lymphocytes infiltrating the gland is likely the predominant pathogenic mechanism, whereas the antibodies are considered by some to be a result of tissue damage.
Treatment of dogs with AT involves supplementation with sodium levothyroxine daily at a dosage that ultimately brings the T4 level within the normal range. Most dogs respond well to thyroid supplementation. Immunosuppressive therapy is not usually used, because by the time patients are diagnosed, the damage to the gland has been done.

**Autoimmune Diabetes Mellitus**

DM is common in dogs and has an onset usually between 4 and 14 years of age. Female dogs are affected more frequently than male dogs. There is a breed predisposition, suggesting an underlying genetic component. A typical presentation involves polydypsia, polyuria, polyphagia, and weight loss. These signs are comparable with those of human DM patients. Cataract formation is common. Hypoinsulinemia prevents the use of blood glucose by cells and a resultant hyperglycosemia and glucosuria results. Ketosis is a potential complication of untreated DM.28

The pathogenesis of insulin-dependent DM in dogs does not always involve autoimmune destruction of the pancreatic beta cells. Other causes include pancreatitis, infection, and insulin antagonistic diseases. Canine DM most closely resembles DM in humans. The presence of circulating antibodies to insulin and to beta cell antigens has been documented, but the role of autoimmunity in beta cell loss is still under study. One study showed a 50% incidence of antibodies to islet cells in newly diagnosed cases of DM. In human patients, autoantibodies to GAD65 (a 65-kDA form of glutamic acid decarboxylase) and to protein tyrosine phosphatase receptor (IA-2 antigen) have been demonstrated. These are 2 important antigens expressed by beta cells of the pancreas.30 Davison and colleagues31 studied 30 dogs with DM for serologic evidence of autoreactivity to GAD65 or the IA-2 antigen. Using cloned and expressed canine versions of these antigens, they found that 2 of the 30 diabetic dogs had significant reactivity to both antigens, and 2 other dogs reacted significantly to GAD65 and 1 dog reacted to IA-2 but not GAD65. As in the case of antibodies to thyroid antigens, a role in pathogenesis has not been demonstrated.

The breed predisposition for DM prompted a study by Catchpole and colleagues25 on the potential association with MHC genes. Using 530 diabetic dogs and 1000 controls, this group examined DLA associations and found 3 haplotypes associated with DM in dogs. These were DLA-DRB1*009=DQA1*001=DQB1*008. These haplotypes were also common in the diabetes-prone breeds (Samoyed, cairn terrier, and Tibetan terrier) and rare in several breeds in which DM is not common.

**AUTOIMMUNE SKIN DISEASE**

**Discoid Lupus**

More than half of canine patients with SLE have involvement of the skin. The skin lesions are often on the face. The term lupus, Greek for wolf, was originally coined because the facial rash on human patients (butterfly-shaped area of erythema over the bridge of the nose and under the eyes) gave the patient’s face a wolf-like appearance. The lupus rash is also exacerbated by sunlight. Canine SLE patients may have alopecia and erythema in a similar location (see Fig. 1A). DL is an autoimmune disease in which the lesions are similar to those of lupus but in the absence of a positive ANA and without involvement of other body systems. Biopsy of a lupus skin lesion stained for immunofluorescence using anti-IgG or anti-C3 reveals the presence of a band of fluorescence along the dermal-epidermal junction. This lupus band is diagnostic for DL (see Fig. 1B).

When the lesions characteristic of lupus are seen without a positive ANA test and in the absence of involvement of other body systems, the disease is called DL.
are located on the nasal planum but can also occur on ear pinnae and around eyes. The disease is seen not uncommonly in dogs but is rare in cats. The prognosis is more favorable for this form of the disease. Treatment with corticosteroids and protection of the nasal planum from UV radiation by using topical sunscreen is indicated. Often topical glucocorticoids or cyclosporine ointment are sufficient for treatment, but in more severe cases systemic corticosteroids may be needed.

**Bullous Skin Diseases**

Among the autoimmune skin diseases, the pemphigus complex of skin disease is one of the most commonly seen. This disease complex is characterized by the formation of vesicles in the skin; the subsequent rupture of these vesicles creates erosions that leave areas of the skin vulnerable to infection. The underlying pathology is instigated by the formation of antibodies against the cellular adhesion molecule, desmoglein 3. Binding of these antibodies causes the epithelial cells to detach from each other, creating acantholysis. There are several distinct diseases within this complex, some more severe than others. The difference in pathology is based on which epithelial layers are affected by the immune reaction.

The least severe form of the pemphigus diseases is pemphigus erythematosus. This disease affects mainly dogs and is most common in German shepherds, collies, and Shetland sheepdogs. It is uncommon in cats. The lesions are superficial and limited to the nose and around the eyes and ears. The oral cavity is not involved and the lesions are only mildly pruritic.

Pemphigus foliaceus is characterized by acantholysis in the most superficial layers of the epidermis. It is the most common of these diseases and is seen in both dogs and cats. The disease is seen in all breeds and both genders and shows no age predisposition. An increased incidence has been reported in the chow chow and the Akita breeds, however. Lesions are often first seen on the bridge of the nose and around the eyes. The ear pinnae are also affected and the footpads may show hyperkeratosis. In dogs, mucosal involvement does not usually occur. In cats, however, lesions may be seen around the nail beds and around the nipples.

Pemphigus vulgaris is by far the most severe form of the pemphigus complex; fortunately, it is also the rarest form. The autoantibodies are directed to antigens on cells that are near the dermal-epidermal junction. Thus the binding of the autoantibodies and subsequent loss of cellular adhesion triggers acantholysis deep within the epidermis. Lesions consisting of bullae, erosions, and ulcers occur on mucosal surfaces (oral cavity, anus, conjunctiva, and so forth), at mucocutaneous junctions, and on the trunk, particularly in areas of skin-to-skin contact, such as axilla and groin. Systemic signs of illness, including fever, depression, and anorexia, are common.

Diagnosis of the pemphigus diseases involves using dermatohistology and immunofluorescence or immunohistochemistry on biopsy specimens to demonstrate the deposition of antibody at the intercellular sites (Fig. 2). The presence of honeycomb-type fluorescence after staining with anti-IgG fluorescein is characteristic of the pemphigus complex.

On dermatohistology, pathologists recognize the presence of superbasilar clefts and vesicles in pemphigus vulgaris; in pemphigus foliaceus, the appearance of subcorneal pustules containing neutrophils and acantholytic cells is characteristic.

Treatment of these diseases involves systemic immunosuppressive therapy and antibiotic treatment as needed when secondary infection is present. In pemphigus erythematosus, the mildest form of the disease, often topical glucocorticoid therapy or cyclosporine topical treatment suffices. For the most severe form, high doses of corticosteroids are often supplemented with other immunosuppressive drugs, such
as azothoiprine (dogs only). The prognosis for the milder forms, pemphigus erythematosus, is good, but for pemphigus vulgaris the prognosis is at best fair and often poor. Lifetime immunosuppressive therapy is often required.

Bullous pemphigoid is a rare autoimmune skin disease in which the autoantibodies are directed against the lamina lucida (the basement membrane). Resultant lesions are the result of separation of the epidermis from the dermis. Very fragile vesicles result, which are not usually visualized in tact but rapidly become deep ulcers. Areas commonly affected include the head and neck, ear pinnae, ventral abdomen, and mucocutaneous junctions. The disease has been reported in dogs and cats.  

The nature and role of autoantibodies in the pemphigus diseases has been studied in dogs. In 1 study, 82% of dogs (n = 64) with pemphigus foliaceus had circulating IgG4 antibodies to keratinocytes as demonstrated by IFA on neonatal mouse skin. Serum from normal dogs frequently contained antikeratinocyte antibodies of the IgG4 subclass. Only those sera with IgG4 antibodies were associated with production of characteristic lesions in mouse skin after passive serum transfer. Thus the investigators concluded that IgG4 may be the pathogenic antibody as in the case of human pemphigus. An earlier study had shown that circulating anti–desmoglein 3 IgG antibodies capable of dissociating keratinocytes are present in dogs with PV. In 1 case study, the potential usefulness of serial IFA titers for antidesmoglein antibodies to demonstrate the effectiveness of therapy was illustrated. A progressive drop in antibody titers was associated with clinical improvement.

**AUTOIMMUNE DISEASES OF THE MUSCULOSKELETAL SYSTEM**  
**Myasthenia Gravis**

Myasthenia gravis (MG) is a disease that causes abnormal weakness and fatigue. It is seen in humans, dogs, cats, and ferrets. In dogs, there is a congenital syndrome...
with this name, but the acquired form has an autoimmune pathogenesis. The underlying problem for both forms is a lack of postsynaptic nicotinic acetylcholine receptors; in the congenital form, the deficiency is due to a genetic mutation, whereas in the acquired form, the loss of acetylcholine receptors is a result of autoimmune attack. Formation of autoantibodies against these receptors triggers receptor degradation. The antibodies also block receptors so that acetylcholine released in the neuromuscular junction cannot bind to its receptor. Ultimately, the IgG antibodies fix complement (type II hypersensitivity) and cause receptor destruction. Thus, the nerve impulse carried to the muscle by acetylcholine does not transmit and the muscle is unable to contract. The degree of receptor destruction affects the severity of the weakness. An affected dog may present with muscle weakness of the skeletal system, but difficulty in swallowing and ultimately in breathing occurs when the disease progresses to involve muscles of mastication and respiratory skeletal muscle. Facial paralysis and megaesophagus are common and many dogs present with only these focal signs.

There seems to be a genetic predisposition for this disease in dogs and cats, although the disease is seen in many breeds. A retrospective study of more than 1000 cases showed that breeds with the highest risk of MG were Akita, terriers (except for Jack Russell), German shorthaired pointers, and Chihuahuas. A similar survey of feline cases revealed that there is a breed predisposition for acquired MG in Abyssinians (and related Somalis). There is a reported association with malignancy of the thymus in some cases. In cats, acquired MG is sometimes associated with the presence of a cranial mediastinal mass.

Diagnosis can be enhanced by observing the response to treatment with anticholinesterase drugs (such as edrophonium chloride), because these drugs allow the acetylcholine to remain longer in the synapse facilitating binding to those receptors that remain intact. Long-term use of these drugs is also suggested for patient management. Testing for the presence of serum antibodies specific for the postsynaptic nicotinic acetylcholine receptors provides confirmation of the diagnosis for this disease. Upright feeding is recommended for dogs with megaesophagus. Surgical removal of thymomas, if present, is often performed. As in other autoimmune diseases, the use of immunosuppressive drugs can prevent further deterioration by halting the further destruction of acetylcholine receptors.

A recent study has determined the nature of the antigenic epitope targeted by antiacetylcholine receptor antibodies in human MG. The main immunogenic region is a conformation-dependent epitope on the extracellular apex of alpha-1 subunit of the muscle nicotinic acetylcholine receptor. This epitope was reported to be recognized by human, canine, and feline antiacetylcholine receptor antibodies.

Rheumatoid Arthritis

Since the late 1970s, a syndrome in dogs similar to RA of humans has been recognized. In a signature case, a miniature poodle presented with chronic hind limb lameness. The presence of a polyclonal gammopathy, elevated leukocyte counts in the absence of infection in joint fluid, radiographic evidence of joint space narrowing in the carpal joints, and areas of subchondral lucency were compatible with the diagnosis of RA. In addition, a test for rheumatoid factor was positive. The dog was treated with prednisone with good response to long-term therapy. This presentation is classic for the disease. The presence of joint stiffness, often first thing in the morning or after inactivity, is often accompanied with depression and anorexia. There is symmetric swelling of affected joints. RA must be differentiated from polyarthritis.
associated with SLE; animals with the former condition are negative for ANA whereas the latter are positive. Radiographic findings in RA generally show far more joint destruction with subchondral bone loss, whereas joints affected in polyarthritis of SLE lack these destructive lesions.

It has been recognized for some time that proinflammatory cytokines are important in pathogenesis of the disease. Recently, the role of tumor necrosis factor α (TNF-α), interleukin 6 (IL)-6, and IL-1 in synovial fluid in pathogenesis of RA has formed the basis for new therapies based on blocking the effects of these cytokines. Clinical trials using antagonists of IL-1, TNF-α, and IL-6 receptor blockers have shown encouraging results in human patients. One study on canine RA patients showed a 30-fold increase of matrix metalloprotease-3 over its inhibitor, tissue inhibitor of matrix metalloprotease-3, in RA dogs when compared with dogs with ruptured anterior cruciate ligament. This pattern correlated with levels of IL-1, IL-12, and transforming growth factor β. It is likely that Th17 cells play a role in initiation of the proinflammatory cytokine production. The presence of these cytokines in the joint fluid stimulates cartilage degradation by the metalloproteases. Thus, the lesion is lytic and accompanied by a synovial inflammation with lymphocyte and neutrophil accumulation, resulting in a fibrous vascular network, called pannus.

The diagnosis of RA includes finding evidence of autoantibodies in serum or synovial fluid. The classic RA factor is an autoantibody directed against a self-immunoglobulin; IgG is most common (for assay details, see later discussion). Other autoantibodies may be directed to type II collagen and glycosaminoglycans. These autoantibodies are thought to have a role in joint destruction.

As with other autoimmune diseases, there are genetic predispositions for RA. In humans, there is an association of HLA-DRB1 with RA. Ollier and colleagues sought to examine canine RA patients to see if a similar predisposition occurs in dogs. They found that several DLA alleles were associated with an increased risk of RA. These are DLA-DRB1*002, DRB1*009, and DRB1*018.

The nature of the initiating cause of RA is not clear in humans or in dogs. There is an association, however, with several infectious disease agents in both species. In the dog, immune complexes consisting of canine distemper virus and anticanine distemper virus antibodies were present in joint fluid of RA patients. Borrelia burgdorferi has also been implicated in RA in 2 dogs recovering from Borrelia infection that were rheumatoid factor positive and progressed to RA.

The prognosis for RA in humans and dogs is not good, particularly if the disease has progressed to joint destruction when therapy is instituted. Current anticytokine therapies show promise in human patients with RA. These include infliximab (a monoclonal antibody to TNF-α) and etanercept (a recombinant TNF-α receptor). Clinical trials with these new immunomodulatory preparations in dogs have yet to be published. In dogs, nonsteroidal anti-inflammatory drugs are often used initially, with corticosteroids, and more aggressive immunosuppressive therapy with methotrexate and gold salts is reserved for the most severe cases.

AUTOIMMUNE DISEASES OF THE EYE
Canine Uveodermatologic Syndrome (Vogt-Koyanagi-Harada Syndrome)

Vogt-Koyanagi-Harada syndrome (VKH) in humans is associated with an autoimmune attack on melanin containing cells. In dogs, the production of autoantibodies against uveal melanocytes results in granulomatous panuveitis and loss of skin and hair pigmentation. The condition is rare in dogs but is seen most frequently in the Akita breed. A recent study of canine VKH showed an association with increased frequency
of DQA1*00201 in the Akita breed. Other affected breeds include the Samoyed, chow chow, Siberian husky, Irish setter, old English sheepdog, and several other breeds.

The presenting signs of VKH are usually ocular, with acute onset of anterior uveitis, keratic precipitates, hyphema, and diminished pupillary reflex. Dermatologic signs may occur concurrently or slightly later than those affecting the eye. A well-demarcated symmetric depigmentation of the nose, lips, and eyelids is characteristic. Treatment of VKH involves vigorous ocular therapy to prevent blindness. The use of topical glucocorticoids and 1% atropine in the eye is accompanied with systemic immuno-suppressive therapy with oral prednisone or methylprednisolone. If there is no or little response, then cyclosporine and oral azathioprine or cyclophosphamide can be used. Lifetime therapy is usually required.

Investigators attempted to reproduce the lesions of VKH experimentally by immunizing Akita dogs with peptides derived from tyrosinase-related protein 1. The resulting autoimmune disease was similar to the spontaneous disease in Akitas. This type of study may lead to better definition of the autoantigens important in VKH in humans and dogs.

LABORATORY METHODS FOR DETECTION OF AUTOIMMUNE DISEASE

**Antinuclear Antibody Testing**

ANAs are antibodies with specificity for nucleic acids and nucleoproteins. They are found in serum of people and animals. Although these antibodies are not normal, low levels are sometimes found in older patients or transiently in patients post trauma. Some infections can induce development of a positive ANA. High serum levels (titers >100), however, are associated with autoimmune diseases. Detection of ANAs in serum is an important parameter for making a diagnosis of SLE.

The most common method for detection of ANAs is an IFA. Green fluorescence of nuclei from a human hepatoma cell line (HEP-2 cells) is present after fixation to permeabilize the cells and subsequent incubation with serum from the patient. Patient serum antibodies are identified after further incubation with a fluorescein-tagged reagent that detects dog IgG. It is customary to initially test sera at a 1:20 dilution. If positive results are seen, then serial dilutions are tested to determine a titer. The titer is determined by looking for the last dilution of serum that gives a positive nuclear fluorescence comparable to the positive control serum.

In addition to a titer, the positive ANA test provides a description of the pattern of fluorescence. The speckled and homogeneous patterns are seen in cases of canine SLE and related syndromes. Other patterns include nucleolar and rim staining, which are less commonly seen.

Detection of ANA is sensitive but not a specific test for autoimmune disease. In 1 study on *Leishmania infantum*, antihistone antibodies were found in 39% of dogs without glomerulonephritis and 88% with glomerulonephritis. In this study, there was a positive correlation between serum creatinine levels and antihistone titers. Other studies have demonstrated high titers of ANA in dogs infected with vector-borne agents: *Ehrlichia canis*, 17% of seroreactors, and *Bartonella vinsonii* (berkhoffii), 75% of seroreactors. In addition, treatment with certain drugs can induce development of ANA in some patients. Implicated drugs include griseofulvin, penicillin, sulfonamides, tetracyclines, phenytoin, and procainamide.

Detection of ANA by other assay methods is reported in the literature. The IFA using *Crithidia luciae*, if positive, indicates that antibodies to double-stranded DNA are present, because the kinetoplast of these protozoa contains only double-stranded DNA. In humans this is the best assay for ANAs in SLE. Other antigens in the nucleus can be detected using enzyme-linked immunosorbent assay (ELISA) or immunoblot. In
1 study, the presence of antibodies to the Sm nuclear antigen and ribonuclear protein was demonstrated.\textsuperscript{54} Double immunodiffusion testing to demonstrate precipitating antibodies specific for nuclear antigens has been applied to canine serum samples. In 1 study, the presence of precipitating antibodies to ribonuclear protein and Sm antigen was associated with the speckled pattern of immunofluorescence.\textsuperscript{65} A recent follow-up study used a line blot assay (Inno-Lia ANA), commonly used in human diagnostics, to evaluate nuclear antigen specificities in ANA-positive dog sera. Antibodies to small nuclear ribonuclear protein antigens were detected in 6 of 20 ANA-positive canine sera, and 2 of the samples reacted with SMB antigen.\textsuperscript{66} Thus it seems that some of the newly developed diagnostic test kits used in human diagnostic laboratories will be applicable to canine patients. There is less information available on the specificity of ANA in cats.

The usefulness of the ANA test in dogs with SLE has been demonstrated in clinical cases diagnosed of high (eg, 1:3200) titers that have been followed over time during treatment with immunosuppressive drugs. It is not uncommon to see the titer fall to negative or near negative (1:40) after chronic treatment.

**DETECTION OF ORGAN-SPECIFIC AUTOANTIBODIES**

Diagnosis of organ-specific autoimmune disease involves the detection of cell specific autoantibodies. For some diseases, such as IMHA, this is a common procedure, with the Coombs test serving as a gold standard. The Coombs test is the primary diagnostic test for IMHA. A recent study by Warman and colleagues\textsuperscript{67} compared the value of using a polyvalent versus a monovalent Coombs reagent. They found that when erythrocytes were screened with anti-IgG, anti-IgM, and anti-C3 separately at both 4°C and 37°C, the test became significantly more sensitive than screening at 37°C with polyvalent Coombs reagent.

For other diseases, such as DM, the detection of antibodies binding to pancreatic islet cells is not a common procedure. Some of the more specific tests are not performed in all veterinary diagnostic laboratories but require that samples be sent to specialists in the area. For example, the assay for the detection of autoantibodies to the postsynaptic acetylcholine receptors in cases of MG is performed primarily at University of California, San Diego (laboratory of Dr G.D. Shelton). In general, the most common method for detection of tissue-specific antibodies is IFA or immunoperoxidase using patient sera on normal homologous tissue. Direct immunofluorescence or immunoperoxidase staining is often used on biopsy samples from tissues with suspected immunoglobulin binding or complex deposition. Hence, in skin from SLE- or DL-affected dogs, the incubation of a biopsy from patients with fluorescein isothiocyanate or enzyme-labeled antisera specific for canine IgG can reveal the presence of bound antibody at the dermal-epidermal junction. Kidney biopsy samples from SLE patients with kidney involvement when stained with similar reagents reveal the deposition of immune complexes. The use of antisera specific for the C3 can also be used to detect immune complex deposition.

**THERAPEUTIC APPROACHES TO AUTOIMMUNE DISEASE**

Autoimmune disease is caused by an uncontrolled immune response against self-antigens. The paramount concern is to dampen this response so that tissue damage ceases. Immunosuppressive therapy is thus a critical element in a therapeutic regimen. Each disease discussion in this article has referred to immunosuppressive therapy. Some of the common immunosuppressive medications are listed in Box 1. Often a combination of these medications is used for optimum effect (eg, prednisone
and azathioprine). The total therapy for each disease, however, is determined by the nature of the disease and the organ systems affected. For example, patients with IMHA in hemolytic crisis must be managed to control the anemia and stabilize the condition in conjunction with institution of the immunosuppressive therapy. Choice of drug and dosage is dependent on whether or not the object is to initiate remission, maintain remission, or rescue form acute crisis. The criteria and appropriate dosing information are presented in the textbook on internal medicine edited by Nelson and Couto. Management of autoimmune skin diseases includes not only systemic immunosuppressive therapy but also topical and often systemic antimicrobial therapy that may be required.

Some autoimmune diseases can be traced to the use of particular medications (such as a sulfinamides and propylthiouracil). In such cases, immunosuppressive therapy is coupled with discontinuation of the causative medication. The prognosis for clinical improvement and eventual discontinuation of the immunosuppressive therapy is good in these cases. Treatment of hyperthyroid cats with propylthiouracil causes a Coombs-positive and ANA-positive syndrome in some cats. This lupus-like syndrome is characterized by lethargy, weight loss, lymphadenopathy, and anemia. In 1 study, more than half of a group of normal healthy cats treated with 6-propylthiouracil (150 mg daily) developed the syndrome. In the majority of the cats, the clinical and serologic signs resolved within 4 weeks of discontinuation of the medication. Those cases for which there is no instigating cause (unfortunately, the majority of cases) are often held in remission by chronic low dose use of an immunosuppressive drug, such as prednisone.

### SUMMARY

There are many autoimmune diseases recognized in humans; many of these have counterparts described in companion animals. The diseases discussed in this article do not constitute the entire spectrum of autoimmune disease in these species. They are the common and better-described diseases of dogs and cats that have a well-documented autoimmune etiology.

There are myriad autoimmune diseases that affect humans; it is likely that similar diseases yet unrecognized in companion animals will be characterized by astute clinicians in the future. The role of genetics in predisposition to autoimmunity is a common characteristic of these diseases in humans and animals. Likewise, the suggested role...
of environmental or infectious agents as instigators is another commonality between humans and the pets that share their environment.

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