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A review of immunologic diseases of the dog

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Abstract

The following review is based on notes used in the teaching of clinical immunology to veterinary students. Immune diseases of the dog are placed into six different categories: (1) type I or allergic conditions; (2) type II or auto- and allo-antibody diseases; (3) type III or immune complex disorders; (4) type IV or cell-mediated immune diseases; (5) type V conditions or gammopathies; and (6) type VI or immunodeficiency disorders. Separate discussions of transplantation immunology and the use of drugs to regulate unwanted immune responses are also included. © 1999 Elsevier Science B.V. All rights reserved.

1. Introduction

Immune diseases have been classified in several different ways, but for the sake of the following review, they will be categorized according to their basic pathogenesis. Six distinct categories can be constructed, the first four being originally described by Gel and Coombs (Types I–IV reactions) (Coombs and Gell, 1975), and the last two (Types V and VI) being added by the author. These six categories include:

1. Type I diseases mediated by homocytotropic antibodies and typified by immediate-type hypersensitivity, i.e., allergies;
2. Type II diseases mediated by auto- or allo-reactive antibodies;
3. Type III diseases mediated by immune complex deposition and characterized by vasculitis;
4. Type IV diseases mediated by cell-mediated immune reactions and manifested by plasmacytic/lymphocytic infiltrates;
5. Type V diseases manifested by gammopathies; and
6. Type VI diseases manifested by immunodeficiency and opportunistic-type infections.

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This classification scheme, while admittedly an oversimplification, has proven very useful in the training of veterinary students. Modifications have to be made for some disorders, such as systemic lupus erythematosus (SLE), which has elements of autoantibody and immune complex reactions. The immune form of amyloidosis also does not clearly fit into any of the six categories, but is included with immune complex disease, to which it bears both immunopathologic and clinical similarities.

It must be stressed that immune diseases are not due to a distinct and pathologic type of immune response; rather, they are the result of normal types of immune responses that occur in a dysregulated fashion, for an excess duration, in an abnormal location, or against antigens that are not normally considered to be foreign. Type I (allergic) diseases are associated with the IgE system, which is one of the primary defenses against parasite attacks. Type II (auto- and allo-antibody reactions) involve the formation of antibodies that react against self-antigens. Type III reactions involve the deposition and clearance of immune complexes, which are essential features of normal immune responses. Type IV diseases involve cellular immunity, which is the most important immune defense against pathogens or pathogenic states involving cells. Type V diseases involve excessive production of immunoglobulins or parts of immunoglobulins, while Type VI diseases occur when something impedes normal immune defense mechanisms, either innate or adaptive.

There are six general factors that underlie most immune diseases of dogs. The foremost is genetic susceptibility. About 60% of dogs are now purebred, and purebreeding always involves inbreeding. Because of the great diversity of immune mechanisms, and hence the genetic complexity of its development and regulation, it is understandable how inbreeding can result in a spectrum of disorders ranging from dysregulation of the immune system and autoimmunity at one extreme to cancers at the other. Although it may be oversimplistic, the susceptibility to autoimmune diseases is thought to be controlled by the interplay of environmental and heritable factors; the latter involving genes of the major histocompatibility complex (MHC) (Campbell and Milner, 1993).

The gender of the dog is the second most common cofactor (Quimby et al., 1980); intact females have the highest incidence of immune diseases and intact males the lowest, as has been observed in humans. The gender bias is not nearly as obvious with widespread spaying and castration. Ovariohysterectomy reduces the incidence, while castration increases the incidence, thus tending to equalize gender effects in neutered animals.

The third most common factor underlying immune diseases is the presence of other immunologic disorders. If a certain breed or bloodline of dogs suffers from one immune disease, they will also have an increased incidence of a wide range of other immunologic disorders, as demonstrated in a study of the Old English sheepdog (Day and Penhale, 1992). Plasmacytic/lymphocytic thyroiditis and hypothyroidism is a common occurrence among dogs that develop other types of immune diseases. Dogs with systemic lupus erythematosus may also present with autoantibody associated cytopenias.

The fourth most common cofactor is infectious diseases. Infections can trigger allergies, the formation of autoantibodies (Adachi et al., 1992), immune complex diseases, cell-mediated pathologies, gammopathies, or immunodeficiencies.

The fifth most common factor underlying immunologic diseases in dogs is drugs. Drugs, including biologics, especially when given to dogs with certain genetic predispositions, can trigger a variety of immune reactions (Cribb, 1989; Giger et al., 1985; Grondalen, 1987; Harvey, 1987).

The sixth most common factor associated with immune disease is cancer (Day, 1996a, b, c). Cancers frequently arise from cells of the immune system or affect the immune system through paraneoplastic effects.

2. Type I (allergic) diseases

2.1. Mechanisms of type I (allergic) disorders of the dog

Type I diseases are mediated by immediate-type hypersensitivity responses occurring in one or more target tissues. Immediate-type hypersensitivity reactions are mediated mainly by IgE, and to a lesser extent IgG, antibodies. IgA and IgM antibodies are only occasionally involved. Antibodies that participate in this reaction have the ability to sensitize cells, in particular mast cells and basophils. Sensitization is a process whereby specific cytotropic antibodies bind to the cell membrane. The binding of the corresponding antigen to these membrane bound antibody molecules triggers the allergic reaction.

The cytotropic antibody system plays an important role in normal immune responses, particularly against parasites. It is somewhat analogous to the secretory (IgA, IgM) antibody system. It is localized to areas of the body where parasites are most apt to gain access or traverse, i.e., in the skin, mucous membranes of the GI and respiratory tracts, in the bloodstream, and around blood vessels in the porto-mesenteric system. The beneficial nature of this response is important to keep in mind; many people believe that cytotropic antibodies and immediate-type hypersensitivity reactions are only of nuisance value.

Antibodies that participate in type I reactions are either homo- or hetro-cytotropic. Homocytotropic antibodies will sensitize animals of the same species but not other species. Homocytotropic antibodies are usually of the IgE class in all animals tested. Heterocytotropic antibodies are usually of the IgG, IgM, or IgA class and will sensitize cells of other species as well.

Cytotropic antibodies have an extremely high avidity for receptors on the membranes of mast cells. Within 3–72 h of their formation, they bind by their Fc portion to mast cells. IgG (and IgA and IgM) cytotropic antibodies have a short life span on the mast cell membrane (12–24 h), while IgE antibodies remain strongly attached for three to six weeks. Cytotropic antibodies are produced in lymphoid tissues underlying the mucous membranes of the body (Peyer's patches, tonsils, diffuse lymphoid aggregates).

Antigens that elicit specific cytotropic antibody responses are called *allergens* (Marsh and Norman, 1988). Allergens generally have a molecular weight restricted to 10 000–70 000 Da. Substances <10 000 Da are not sufficiently large to bridge two adjacent membrane bound molecules of cytotropic antibody, while larger allergens will not penetrate the mucous membranes. Allergens are highly polar substances containing many sulfhydryl groups and will sensitize in nanogram to microgram amounts. Besides their

size and chemical makeup, allergens that elicit IgE responses are often taken up by the host at low dosage and across some mucosal surface (Marsh and Norman, 1988). Allergens can be parts of parasites, such as house dust mites or cockroaches. Conditions which allow an excessive build-up of these insects in the environment will also increase the exposure level to their allergens. Many allergens resemble proteinaceous substances on the cuticle of parasites (e.g., pollen, mold spores, dander). Drugs, cosmetics, and substances in cigarette smoke may also act as allergens.

The target cells of allergic reactions are the tissue mast cells and circulating basophils. There are approximately 7000–20,000 mast cells per µl of skin or intestinal tissue. Mast cells and basophils possess cytoplasmic granules that contain potent inflammatory mediators and anticoagulants, such as heparin, histamine, serotonin, kinin protease, and slow reacting substance of anaphylaxis (SRS-A). They also contain substances that have reparative functions such as chymase, hyaluronic acid and platelet aggregating factor, as well as eosinophil and neutrophil chemotactic factors. SRA-A is not a single compound, but a mixture of spasmogenic and vasodilatory metabolites of arachidonic acid called leukotrienes. SRA-A is also not a sole product of mast cells, but a contribution from both mast cells and other activated tissue mononuclear cells. Basophils are probably a circulating form of mast cell, although they differ in their responses to certain substances; cromolyn sodium inhibits release of mast cell granules but not basophil granules.

The release of mast cell or basophil granular contents is triggered by the binding of allergen to specific cytotropic antibodies on the cell surface. Binding of one molecule of allergen to two adjacent molecules of specific cytotropic antibody alters in the cell membrane and initiates energy dependent processes that culminate in the release of the pharmacologic mediators of the allergic response. In any given individual, the degree of sensitivity is directly proportional to the amount of histamine released by mast cells or circulating basophils in response to a standard amount of a particular allergen.

The IgE system is under strong T-cell regulation (Romagnani, 1994), and there is an inverse relationship between cellular and IgE immunity. IgE production is enhanced by thymectomy, sublethal whole body irradiation, anti-thymocyte serum, radiomimetic drugs, certain viral infections, and in individuals with congenital and acquired immunodeficiency syndromes involving cellular immunity (Wiskott–Aldrich syndrome, ataxia–telangiectasia, Hodgkin's disease).

The phenomenon of allergic breakthrough occurs has great clinical relevance. IgE production is minimal in normal non-allergic individuals and is kept that way by internal regulatory mechanisms involving T-lymphocytes (Romagnani, 1994). Natural events, such as viral infections, can temporarily depress T-cell-mediated immunity and thus normal dampening mechanisms are removed and enhanced IgE production occurs. Genetically predisposed dogs developed higher levels of specific IgE when exposed to pollen extracts several days following canine distemper immunization (Frick and Brooks, 1983). Enhanced production of cytotropic antibodies to non-parasite antigens may also result from disuse of normal IgE mediated immunity. Heavily parasitized people may have less problems with nuisance type allergies (Buckley et al., 1985), indicating that an IgE immune system engaged in normal parasite defenses is less likely to react to peripheral or related antigens.

The development of allergies is also influenced by genetics (Huang and Marsh, 1993). If both parents have atopy, 75% of their children will be atopic. If one parent has atopy, the chances are 50%. Similar genetic predispositions have been seen in animals, especially among purebreds.

2.2. Specific Type I immune diseases

2.2.1. Systemic anaphylaxis

Anaphylaxis can be classified as systemic (anaphylactic shock, angioedema, hives) or localized (GI, skin, respiratory allergies). Systemic anaphylactic reactions are triggered by allergens that enter the bloodstream and react with specifically sensitized mast cells in the target organs. Systemic reactions of this type can be provoked in dogs, by routine vaccines, drugs (Phillips et al., 1998), foreign protein injections, insect bites, food stuffs, or pollens and molds. In spite of the widespread belief that anaphylactic reactions often escalate in severity with repeated sensitization, severe anaphylactic reactions can occur without known previous exposure and are much more likely to manifest themselves similarly following repeated elicitation than to escalate in severity.

2.2.1.1. Anaphylactic shock. Anaphylactic shock is the most severe form of systemic anaphylaxis. The target organs in dogs tends to be the porto-mesenteric system, and unlike humans, the smooth muscles of the lungs are either spared or mildly involved. The local release of vasoactive amines results in porto-mesenteric or splanchnic vasodilatation, venous pooling of blood, peripheral vasocollapse, and in severe cases, death. Clinical signs include nausea, vomiting, diarrhea, ataxia, coldness and pallor of mucous membranes, rapid breathing, and in some cases, bronchial constriction (asthmatic type breathing). Death may occur in as short as 5 min. Animals living more than 5 min may also show signs of hypersalivation, tenesmus, and defecation. Profuse salivation may be seen after 10–20 min.

2.2.1.2. Angioneurotic edema. Angioneurotic- or facioconjunctival-edema is a less severe systemic allergic reaction manifested by swelling of the lips, eyelids, conjunctiva, and occasionally associated with mild bronchial constriction, GI signs, and mild shock. Edema reaches a peak in 10–30 min and may take several hours to subside. This type of reaction is evoked by the same sorts of allergens as systemic anaphylactic reactions.

2.2.1.3. Urticaria. Urticarial reactions (hives) are coin-like, sharply delineated, and highly pruritic lesions of the skin that occur within minutes of allergenic exposure. They are analogous to the lesions evoked by intradermal inoculations of histamine. Hives may be the sole manifestation of allergic reaction or may be associated with any of the above reactions. Clinical signs generally last for 1/2 to 2 h and then subside.

Most dogs will only manifest anaphylactic reactions after provocative testing (IV, IM, subcutaneous injection of suspected allergen), something which is potentially dangerous and not usually done. Fortunately, the diagnosis can usually be made from a detailed history.

The most effective control of systemic allergic reactions is to avoid re-exposure to the offending allergen. This is easily done when drugs are the offenders, because alternative products are usually available that are not allergenic. However, vaccines, which are the most common cause of such reactions, are more problematic for veterinarians. Many veterinarians believe that booster vaccinations are absolutely essential and are unwilling to forego their use even when they have previously evoked an allergic reaction. If systemic allergic reactions to vaccination are life-threatening (i.e., anaphylactic shock), the risk of subsequent immunizations will far out-weigh the negligible benefits provided by booster vaccinations. The chances of an older and well-vaccinated dog getting potentially fatal infections like canine distemper, infectious canine hepatitis, parvovirus enteritis, leptospirosis or rabies are extremely low, and the other vaccinatable diseases, such as kennel cough, parinfluenza, Lyme borreliosis, and coronavirus enteritis are of limited clinical relevance in the general dog population. Even concern about the legal requirement for rabies boosters should not override common sense; a waiver can usually be obtained for rabies vaccinations when the use of such vaccine is life threatening. If systemic allergic reactions to vaccines are severe, but not life threatening (hives, angioneurotic edema), pretreat the animal 1 h before immunization with antihistamine and prednisolone. Give 1/10th the vaccine dose; if no adverse reaction is seen in 10–20 min, administer the remainder. Changing brands or types of vaccine will seldom prevent the problem, because most vaccines contain similar offending allergens. However, leptospirosis bacterin is the worst offender and the least essential vaccine, and can easily be left out of the vaccination regimen.

2.2.2. Localized allergic reactions of specific organ systems

2.2.2.1. Allergic respiratory diseases. Allergic rhinitis (vasomotor rhinitis) is the most common allergic disorder of people, but is infrequent in dogs. Inhalant allergens in dogs are more commonly associated with atopic dermatitis than with rhinitis, while the opposite is true for people. This might reflect a difference in distribution of sensitized mast cells in the two species. It is caused by inhalation of pollen, danders, dusts and molds, cosmetics, cigarette smoke, and miscellaneous inhalants. Clinical signs may be seasonal in the case of seasonal allergens, or year around.

Sneezing and snuffling (reverse sneezing) are the most common presenting clinical signs of allergic rhinitis. Signs are usually seasonal and last for a few weeks. During the allergic period the clinical signs are usually intermittent rather than persistent. Nasal discharge is scant and usually serous in nature. In severe cases, however, sneezing and nasal discharge can be pronounced. Conjunctivitis, with reddening of the eyes and serous ocular discharge, is an uncommon accompanying feature.

Before making a diagnosis of allergic rhinitis in a dog, it is important to rule out other causes of chronic rhinitis. Nasal mites, if undetected, can present in an identical manner, and indeed, only those dogs that become allergic to their nasal mites will show clinical signs of infestation. Nasal aspergillosis may present with sneezing. Neoplasia of the nasal passages, which is relatively common in older dogs, may present initially as sneezing. Foreign bodies, especially grass awns, occur in both dogs and cats and are associated with

violent sneezing in the initial stages and purulent unilateral nasal discharge in the later stages.

Complete blood counts and other routine laboratory tests will usually be normal in dogs with allergic rhinitis. Intradermal allergen testing is of variable benefit in determining either the allergic nature of the rhinitis or the specific allergen or allergens involved. Diagnosis is usually made by ruling out other causes, finding eosinophils in the nasal wash, and response to hospitalization or other changes in the environment. Response to glucocorticoid therapy may also be helpful.

The preferred treatment for allergic rhinitis is to find the cause of the allergy and remove the allergen from the environment. If this is not possible, treat with glucocorticoids using an every-other-day maintenance therapy at the lowest effective dose and limit treatment to periods when disease signs are present.

Allergic Bronchitis is the most common allergic condition of the respiratory tract of dogs. It is presumably due to the inhalation of the same types of substances that are associated with allergic rhinitis. Clinical signs are usually chronic and year around, and manifested by a chronic, dry honking-type of cough. The cough is easily elicited upon tracheal compression, and is often evoked by exercise, drinking of water, excitement, or pulling on the leash. The cough is usually non-productive but occurs in paroxysms that end with a gag or retch. Following allergic stimulation of the tracheal and bronchial mucosa, the mucosal cells secrete a thick and tenacious type of mucus. This mucus, though not copious, is very thick and tenacious and difficult for the animal to clear. The characteristic cough is due to low grade irritation and from the animal's attempt to clear mucus from the airways.

It is essential to differentiate this disorder from other causes of chronic bronchitis. In toy breeds, especially poodles, the collapsing trachea syndrome can produce the same signs. To further complicate matters, allergic bronchitis and collapsing trachea syndrome may coexist. The inability of dogs with collapsing tracheas to clear foreign matter from their airways may predispose them to the development of allergies in the tracheobronchial tree. Chronic bronchitis due to low grade bacterial infections is common in older dogs, especially larger sporting breeds. This is probably associated with a degeneration of the local mucocillary activity of the bronchial mucosa. Lung worms, foreign bodies, primary lung tumors, congestive heart failure, and disorders associated with tracheal compression should also be ruled out. Kennel cough due to various infectious agents, in particular *Bordetella bronchiseptica*, is also an important differential diagnosis.

The diagnosis of allergic bronchitis is fairly easy to make. Other causes of chronic bronchitis can usually be eliminated by the history, physical exam, and chest radiographs. Once other causes are eliminated, the diagnosis can be confirmed by analysis of the tracheal wash. The tracheal wash is usually bacterially sterile and contains numerous eosinophils. The CBC is normal, or occasionally shows a mild eosinophilia. Chest radiographs may be normal or show a mild increase in bronchial markings.

It is very difficult to find the cause of the allergy or to avoid exposure. The condition is usually treated with expectorant/bronchial dilator drugs such as theophyline elixir with either potassium iodide or glycerol guiacholate, or with glucocorticoids, or both. Mild cases will respond very well to the expectorant/bronchial dilators alone, or to low dose glucococorticoid therapy.

Eosinophilic pneumonitis is relatively common in dogs and resembles the human diseases referred to as acute eosinophilic pneumonia (Pope-Harman et al., 1996), pulmonary eosinophilias (Bain and Flower, 1996), or 'pulmonary infiltrates with eosinophilia' (PIE). Pulmonary eosinophilias are a diverse group of disorders in people and are characterized by pulmonary infiltrates, rich in eosinophils, and peripheral eosinophilia. Known causes include fungi, especially *Asperigilla fumigatus*, parasites, toxins, and drugs. The causes in dogs are similar and at least twofold: (a) hypersensitivity reaction associated with the inhalation of allergens, in particular molds, or the use of certain drugs; and (b) parasite migration (occult dirofilariasis, lung worms, ascariasis in young animals). Idiopathic forms of pulmonary eosinophilia in humans include Loffler's syndrome, acute and chronic eosinophilic pneumonia, hypereosinophilic syndrome, bronchochentric granulomatosis, and pulmonary eosinophilia associated with vasculitis (Bain and Flower, 1996). Most of these idiopathic forms also occur in dogs, but are uncommon.

Clinical signs of eosinophilic pneumonitis in dogs include fatigue, exercise intolerance, soft cough, dyspnea. Chronic pulmonary can be associated with weight loss. Chest radiographs show diffuse interstitial pulmonary infiltrates around alveoli and bronchioles. Numerous eosinophils in tracheal washes and in the blood. It is important to rule out parasitic infections.

Occasionally it is possible to associate the disease with environmental allergens, in which case it is best to put the animal in a new environment or alter the existing environment to eliminate exposure. In most cases, however, the cause is unknown and environment control is not feasible. Glucocorticoids for several weeks or more will cause a rapid resolution of radiographic and clinical signs. Disease will often not reoccur following treatment.

Pulmonary granulomas with eosinophilia are uncommon in dogs. The cause is multifactorial and probably involves either a florid reaction in the pulmonary parenchyma to dead or dying parasites, especially dirofilaria, or to idiopathic hypersensitivity angiitis. Clinical signs include a soft cough, dyspnea, fatigue and exercise intolerance, large or multiple smaller circumscribed masses in lungs on radiographs, pronounced eosinophilia in tracheal washes and in the blood. Vascularly orientated granulomatous reaction with intense tissue eosinophilia is usually seen on biopsy. In the case of dirofilaria, dead or dying adult worms might be seen in the lesions. Glucocorticoids are effective in shrinking masses and improving pulmonary function.

2.2.2.2. Intestinal allergies. Intestinal allergies in dogs can be both intrinsic (food intolerance) and extrinsic (allergic) in origin (Roudebush, 1993). Although the triggering mechanism is different in extrinsic and intrinsic gastritis, both forms are ultimately mediated by basophil degranulation and vasoactive substances, clinical signs are virtually identical, and treatment is similar.

Acute allergic gastritic is manifested by vomition of food within 30 min or less of eating, while allergic reactions in the upper small intestine are more apt to be manifested by the vomiting of bile tinged mucus from 30 to 90 min or more after eating. Vomiting may be periodic (2–3 times per week) or more constant (several times a day). Weight loss and poor hair coat are only observed in more severe cases.

Food allergies or intolerances affecting the small intestine are also common in dogs and manifested by intermittent or chronic diarrhea, associated with the ingestion of certain foods. Bowel movements are usually normal in frequency and in amount, but often loose or watery and foul smelling. Most dogs will appear otherwise normal, although in more severe cases, the animals may be thinner than normal and the hair coats dry and of poor quality. Eosinophilia is a variable feature, and even when present, it is seldom pronounced.

Eosinophilic enteritis is a more severe and less common form of intestinal allergy. Diarrhea is more severe than in dogs with allergic enteritis, systemic signs of ill-health more prominent, and eosinophilia a striking finding.

Allergic colitis has been described in dogs (Paterson, 1995). Stools are normal, mucous-laden, or soft. Frequency of defecation is often increased. Weight loss can be a problem in animals with severe colitis. It is important in dogs to rule out other causes of colitis, e.g., whipworms, idiopathic colitis, boxer colitis, spastic colitis (mucous colitis), lymphosarcoma, etc.

Dietary allergies and intolerances are so common in dogs that it is justified to try special diets before undertaking elaborate clinical work-ups. If the diarrhea is particularly severe, fecal blood loss in the form of melena or red blood is present, weight loss is pronounced, other systemic signs are present, or if the animal is hypoproteinemic, then the initial diagnostic work-up should be more complete.

There are no specific tests to diagnose food allergies or intolerances. Allergen testing for food allergies has not been extensively studied. In man, serum histamine levels will increase several hours after feeding allergenic foods. A mild eosinophilia in the absence of parasites may be an additional clue. Ermel et al. (1997) were able to evoke allergic reactions directly on the mucosa of the stomach by gastroscopy, although this is not clinically applicable.

The diagnosis of allergic enteritis is ultimately made by showing a response to a hypoallergenic diet. The ingredients of a hypoallergenic diet in dogs consist of foods not normally found in their commercial diets. Cottage cheese and/or tofu and rice are good starting hypoallergenic diets for dogs. Fish and potato is an alternative hypoallergenic diet (Paterson, 1995); some dogs are allergic/intolerant to milk proteins, so cottage cheese and rice may not always be appropriate. If there is no improvement with the first diet, try an alternative diet for another two weeks. If there is still no improvement, forget about food allergies or intolerances and search for other causes. If there is a dramatic improvement, add a multivitamin and mineral supplement to the diet and continue to feed it for several more weeks. After this time, new foods can be introduced one at a time every two weeks. If the condition is severe, or dietary control is incomplete or impossible, prednisolone is an effective, alternative treatment.

2.2.2.3. Allergic skin disease. Atopic dermatitis is prevalent in dogs and was initially attributed to allergens that were either inhaled or directly contacted the skin (pollens, molds, danders, house dust) or to insect bites (fleas). The role of dietary allergens as a cause of atopic dermatitis in dogs has recently been given greater emphasis (Ermel et al., 1997; Jeffers et al., 1996; Paterson, 1995). There is a breed predisposition, especially to

smaller terriers (e.g., Cairn, West highland white), Golden retrievers, Labrador retrievers, and Dalmatians.

Clinical signs include pruritic lesions about the face, feet, and axilla, often associated with self-mutilation. Intradermal allergen testing is most effective in animals showing skin manifestations associated with inhaled allergens, while dietary allergens are usually identified by the use of elimination/provocation diets (Jeffers et al., 1996). Dogs are fed a food-elimination diet until clinical signs disappear, then challenged with the original diet or to single major components of the original diet (e.g., beef, chicken, eggs, milk, wheat, soy, or corn). Assays detecting allergen specific IgE in serum are increasingly being used to diagnose allergens in dogs.

Treatment should be directed whenever possible to avoiding offending allergens. Desensitization using small dosages of allergens injected parenterally is used in dogs, but the main treatment still is glucocorticoids.

2.2.2.4. Eosinophilic encephalitis. Some dogs, ranging from puppies to adults and presenting with signs of diffuse or multifocal meningoencephalitis, will demonstrate high numbers of eosinophils in their cerebral spinal fluid (Smith-Maxie et al., 1989). Some of these animals have parasitic infections in the brain on post-mortem, while many others demonstrate no obvious cause.

3. Type II immune diseases of the dogs

3.1. Mechanisms of type II immune diseases

Type II reactions are characterized by the development of antibodies that react with cells or cellular constituents. The term 'cytotoxic', which was originally ascribed to this category of immune diseases by Gel and Coombs, is not completely correct because autoantibodies are not only directed against cell surfaces, but also against cell receptors, basement membranes, basal lamina, or intracellular cement substances.

The formation of self-reacting antibodies involves eight basic mechanisms:

1. anomalies of normal immunoregulation, i.e., abnormalities in self/non-self-recognition;
2. alloimmunization;
3. unmasking of hidden or sequestered antigens;
4. immunoglobulin focusing;
5. cross-reacting antibodies;
6. innocent bystander effect;
7. non-specific immunoglobulin focusing; and
8. production of autoantibodies by cancerous pre-B or B-cells.

Although the thymus plays an important role in eliminating clones of lymphoid cells that can react to self-antigens during early fetal development, it is evident that many individuals retain the capacity to react in a humoral or cellular manner against a range of

self-antigens. It has been said that autoantigens are not so abundant that they induce clonal deletion or anergy, but not so rare as to escape recognition entirely (Janeway and Travers, 1996). Corato et al. (1997) concluded that normal dogs had auto-erythrocyte reactive lymphocytes, but that these lymphocytes must be primed in individuals with AIHA or a heritable susceptibility to the disease. Therefore, the capacity to generate self-reactive immunity appears to be suppressed by the normal immune system. The T-cell system is particularly involved in down-regulating autoantibody production by B-cells, and when antibodies against a particular self-antigen are generated, some aspect of T-cell regulation must have failed. Normal T-cell regulatory function can be influenced by a number of conditions, including microbial (especially viral) infections, vaccines (McAnulty and Rudd, 1985), diseases of the lymphoreticular system such as lymphosarcoma, certain drugs, genetic weaknesses resulting in instabilities in self/non-self-recognition, and unrecognized factors.

Alloantibody formation is a second way that self-reacting antibodies can be produced, usually applies to the immunization of mothers to paternally inherited antigens of the fetus, in particular RBC antigens. Sensitization of the mother can occur during pregnancy when fetal blood leaks into the maternal circulation, or at the time of parturition when the maternal–fetal attachments break down. If the mother is immunized against red cell antigens common only to the fetus, cytotoxic antibodies will be passed to the fetus in the colostrum and a condition known as neonatal isoerythrolysis will occur. Sensitization to allogeneic RBCs can also occur as a result of prior blood transfusions from dogs with different RBC types.

Lymphoid cells that are programmed to react against deeply sequestered (i.e., hidden) antigens may escape destruction during the fetal development of the immune system. Indeed, many body proteins are antigenic but are not recognized as such because they are sequestered from the host's immune system (Streilein, 1993). There is also evidence that natural autoantibodies exist to deeply sequestered red cell membrane proteins (e.g., spectrin) in dogs (Barker and Elson, 1993; Barker et al., 1991), and that these antibodies may help in the destruction of aged and worn red cells. Thyroglobulin is immunogenic if it is injected into other areas of the body, and anti-thyroglobulin antibodies are often secondarily associated with primary destructive diseases of the thyroid gland in dog and humans. Following myocardial infarction or hepatic necrosis in humans, antibodies to cardiac muscle, microsomes, and other cellular constituents appear transiently in the serum. Anti-nuclear antibodies (ANA) often appear in the serum of dogs with aural hematomas, and these antibodies probably result form the extravasation and destruction of blood cells (and thus release of sequestered nuclear antigens) into the ear tissues. In these examples, the autoantibodies may be markers of disease (as in hypothyroidism in dogs) and of no clinical significance. The autoantibodies cannot react with antigens that are sequestered deep within the cells. In other cases, however, autoantibodies that are generated in this manner may actually have a pathogenic effect. *Brucella canis* infection often localizes in the testicles of dogs and can induce an autoantibody reaction to sperm and infertility (George and Carmichael, 1984; Serikawa et al., 1984).

Immunoglobulin focusing can occur when certain antigens are arrayed on the surfaces of cells. In the case of certain bacterial, protozoal, rickettsial, and viral diseases, antigens of the microorganisms are incorporated into the cell surfaces of host cells and render the

cell membrane foreign. The exact nature of anemia in such infections is not always straightforward; erythrophagocytosis is a prominent feature of *Babesia gibsoni* infection (Wozniak et al., 1997) and although hemolytic activity is present in the serum, it does not appear that anemia is immune mediated in the classical sense (Onishi et al., 1993). Drugs may also combine with cellular proteins, thus creating new antigens and rendering the cell surface a target for host immunity.

Antigens present on some microorganisms may elicit antibodies that cross react with normal cellular constituents. The classic example in humans is the cross reaction between certain streptococcal antigens and self-antigens on the heart valves.

Innocent bystander reactions occur when circulating immune complexes are adsorbed unto RBCs, WBCs or platelets. Coated cells are then cleared by the phagocytic system. Non-specific immunoglobulin binding is a somewhat similar reaction. Some drugs, in particular cephalosporins, can damage RBC membranes allowing for the non-specific adherence of immunoglobulins and complement.

Lymphoid or plasma cell tumors can sometimes produce monoclonal autoantibodies that react with self-antigens, although this situation is admittedly rare. The likelihood of a malignant B-cell producing an antibody reactive to a self rather than non-self-antigen is very low and the likelihood of an autoantibody being pathogenic would further reduce the odds. A German shepherd dog with malignant myeloma and a polyneuropathy may be such an incident (Villiers and Dobson, 1998).

3.2. Immune-mediated cytopenias

3.2.1. Immune-mediated hemolytic anemia (IHA)

IHA occurs in primary (idiopathic) and secondary forms. Idiopathic IHA is not associated with any known existing cause, while secondary IHA is usually associated with infectious diseases, drugs, or neoplasia. In man and cats, two-thirds or more of the cases of IHA and ITP are secondary in nature. However, most cases of IHA in dogs are 'idiopathic'. In a study of 14 dogs with IHA, only three subsequently manifested underlying disorders (one dog developed SLE, one generalized lymphoma, and one an unidentified thromboembolic disorder) (Day, 1996a, b, c, 1998).

Autoantibodies are usually produced against erythrocyte membrane antigens. In precipitation studies in dogs, antigens were either cell surface associated (glycophorin-like peptides of 29–42 kDa, unknown peptides of 37–100 kDa, phospholipids, or proteins involving the erythrocyte cytoskeleton (Barker et al., 1991).

IHA can be classified in two ways. In human medicine, where much more is known about the diverse causes of these disorders, the classification is as follows:

IMMUNE-MEDIATED HEMOLYTIC ANEMIA

Warm antibody types
    Idiopathic warm IHA
    Secondary warm IHA
        Associated with SLE and other autoimmune disorders
        Associated with lymphoreticular malignancies
        Associated with viral infections

Cold antibody types
    Idiopathic cold agglutinin disease
    Secondary cold agglutinin disease
        Associated with *Mycoplasma pneumoniae* infection, mononucleosis, and other viral infections
        Associated with lymphoreticular malignancies

Paroxysmal cold hemoglobinuria

Idiopathic

Associated with syphilis or viral infections

Although this type of classification also applies to veterinary medicine, the tendency has been to lump all immune-mediated hemolytic anemias into a single group. This is unfortunate because in one study of 42 cases of Coomb's or agglutination positive hemolytic disease in dogs, a wide spectrum of conditions were evident (Klag et al., 1993). About three-fourths of dogs were Coomb's antibody positive (usually IgG without complement); sphereocytosis was observed in two-thirds, hemoglobinemia and hemoglobinuria in 10%; one-third had moderate to severe reticulocytosis, <30% had mild reticulocytosis, and over one-third had a non-responsive anemia at the time of diagnosis. Concomitant thrombocytopenia was seen in two-thirds of the dogs. The mortality also varied greatly; 29% died during hospitalization and the risk of death was significantly increased in animals without marked reticulocytosis, lower PCVs, and greatest elevations in serum bilirubin. It is obvious from these findings that there are a number of different forms of IHA. Therefore, it is preferable at our stage of knowledge to classify IHA in animals in more clinical terms, e.g. peracute/acute, subacute, chronic. It must be recognized, however, that within each of these clinical groups that some of the cases are secondary, some idiopathic, some are drug induced, and that either warm or cold agglutinins may be involved.

3.2.1.1. Peracute IHA. Peracute IHA occurs more commonly in larger dogs of the shepherd type and in animals from 2 to 6 years of age. This form of IHA probably represents the (10–29%) subgroup of dogs described by Klag et al. (1993), which had the highest mortality and associated non-responsive anemia, lowest PCVs, and highest serum bilirubin levels. The etiology of the disease is unknown. Some event, perhaps a subclinical viral or bacterial infection, triggers the rapid development of a high-titered, broadly reactive, hemagglutinin. A similar non-immune type of peracute hemolytic anemia has been observed rarely in dogs and commonly in cats with overwhelming gram-negative bacterial sepsis. The serum hemagglutinin in peracute IHA often reacts against red cells from virtually any canine donor and commonly crosses species lines to agglutinate human, cow, cat, goat, sheep and horse cells. The disorder has no precise human counterpart, but aspects of the canine disease resemble microangiopathic-type hemolytic disorders of man.

Dogs with peracute IHA often manifest malaise, lethargy, and sometimes fever for 1–3 days prior to the actual hemolytic episode. At the time of hospitalization, the dogs are obviously depressed, the PCVs range from slightly depressed to 15% or so, and

hemoglobinuria (port wine urine) is observed in 10% or less of the animals. The packed cell volume will usually drop to levels below 10% within 24–72 h of the time of admission, and the urine and serum will often become increasingly icteric in appearance. Thromboembolic phenomena frequently accompany the peracute anemia and may be clinically manifested by neurologic (vestibular abnormalities, blindness, dementia), cardiopulmonary (respiratory distress, arrhythmias), or renal signs (elevated BUN).

The characteristic feature of this disorder is the presence of 'in-tube' or 'in-saline' red cell agglutination, severe non-responsive anemia (at the time of initial hospitalization), variable thrombocytopenia, negative direct Coomb's test for IgM, IgG, and C3, and laboratory evidence of DIC (variable elevations in FDPs, decreased antithrombin three levels, consumptive-type thrombocytopenia). The spontaneous agglutination of the RBCs does not disappear when the blood is diluted 1 : 4 or more with saline, hence the term 'in-saline agglutination' or 'in-saline agglutinins'. It is extremely difficult in most cases to find compatible blood due to the very high titers of broadly cross-reacting agglutinins. Because a small proportion of animals with similar signs are septic, blood cultures should be a routine part of the clinical work-up.

Affected dogs should be immediately heparinized and aspirinized to treat the DIC and the associated microangioipathic anemia, thrombocytopenia, and thromboembolic phenomena. The production of in-saline agglutinins should be halted with immediate glucocorticoid and cyclophosphamide therapy. Incompatible transfusions should be avoided if at all possible. If it becomes necessary to transfuse, utilize the least incompatible blood, make sure that the dog is fully heparinized prior to transfusion, and give the blood slowly over several hours. If any untoward transfusion signs are noticed, i.e., hemoglobinemia, hemoglobinuria, facial edema, or cardiac problems, immediately discontinue the transfusion. Giving incompatible blood to a non-heparinized dog is counter-indicated because it will greatly enhance the DIC, thromboembolic phenomena, and the microangiopathic red cell destruction. Dogs can tolerate PCVs of 10 or so, providing they are resting comfortably and their hearts are functioning normally. Gradual improvement will occur beginning 48–96 h after treatment is started. This will be first manifested by an improvement in the outward demeanor of the animal and a stabilization of the PCV. The PCV will then rise slowly to levels of 15–20 over the next week or so, the platelet count will return to normal, and autoagglutination will subside. Full restoration of the PCV can take 1–3 months.

Heparinization is usually continued for 10–14 days, aspirin therapy for several months, cyclophosphamide for 2–3 weeks, and glucocorticoids for several months before being discontinued. Recovered dogs do not usually have recurrent bouts of the disease. Unlike other forms of AIHA, the prognosis of peracute disease has to be considered poor. If not treated aggressively, almost all animals will die. A 50% or more mortality is associated even with optimal treatment. Whether the prognosis is grave or poor is somewhat dependent on the following negative prognostic factors: (1) hemoglobinuria; (2) no compatible donors; and (3) severity of thromboembolic signs. Also, if the dog survives the first three days of optimal treatment, the prognosis becomes more favorable. Therapy takes several days to take effect, and the bone marrow in these animals usually has not had enough time to respond by the time the PCV reaches its lowest point (marrow takes a week or so to begin to respond to acute blood loss).

3.2.1.2. Subacute IHA. Subacute IHA is the classical or text-book form of the disease in dogs, i.e., a Coomb's positive, responsive anemia, with spherocytosis (Day, 1996a, b, c, 1998). Subacute IHA accounts for two-thirds or more of hemolytic anemias in the species; it occurs much more often in purebreds than mongrels and disproportionately in Cocker Spaniels compared to other breeds.

Clinical signs occur over a period of 7–14 days or more, usually when the levels of RBC bound antibodies and complement reach sufficient density for the spleen to remove them from the circulation. Intravascular hemolysis is uncommon. Owners are usually not aware of any clinical problems until the hematocrit decreases to a mean of 17.6% (Day, 1996a, b, c). At this point, lethargy, fatigue on exercise, pallor and tachypnea are manifested. Affected animals may show generalized lymphadenopathy, pallor of the mucous membranes, and hepatosplenomegaly upon physical examination. Icterus and bilirubinuria are seen in 10% or so of the cases.

The anemia of subacute IHA is very responsive, with increased MCV, reticulocytosis, and polychromasia. Spherocytes are usually evident on blood smears. Spherocytes are damaged RBCs that have imbibed fluid and become spherical rather than disc-shaped. The WBC count is often elevated, occasionally with a left shift to metamyelocytes. This flamboyant leukocyte response is a 'slop-over' from the intense erythropoiesis and may be mistaken for granulocytic leukemia in some cases. In order of prevalence, Coomb's reactivity is usually associated with IgG, IgG and IgM, or IgM autoantibodies (Day, 1996a, b, c); the Coomb's antibodies are warm-reactive in most cases. Cold agglutinins, when present, are of the IgM class and detectable at 4°C. Autoantibodies in dogs with subacute IHA have been associated with glycophorins similar to those found in the human rhesus complex, unknown surface peptides, and possibly to membrane associated phospholipid (Barker et al., 1991; Barker and Elson, 1995).

Dogs with subacute IHA are not in any immediate danger, so the initial treatment is usually prednisone alone (Day, 1996a, b, c). The initial dosage is 3–4 mg/kg for small dogs and cats, 2–3 mg/kg for medium-sized dogs, and 2 mg/kg for large dogs. This dose is often divided twice daily for the first few days. The hematocrit should stabilize within seven days and start to rise by 10 days or so. Remission in good responders is usually achieved within one month (Day, 1996a, b, c).

If there is a poor response by week 2 of treatment, several weekly cycles of cyclophosphamide (50 mg/m^2 per os daily for four consecutive days of each week) should be added to the treatment regimen. The dose of prednisone should be tapered to 1 mg/kg by weeks 3–6, depending on the rate of disease remission. After the hematocrit has returned completely to normal, the prednisone dosage is halved to 1 mg/kg every other day. It is kept at that level for 1–4 months, depending on how fast initial remission was achieved (the longer it takes to achieve remission, the slower therapy is withdrawn).

The prognosis is good in idiopathic cases, with a mortality of <15% (Day, 1996a, b, c). Relapses are uncommon in dogs with idiopathic (primary) IHA. However, if the IHA is secondary to other problems, the long-term prognosis is poorer (Day, 1996a, b, c).

3.2.1.3. Chronic IHA. Chronic IHA is relatively uncommon compared to peracute and subacute forms. The anemia develops over weeks or months and presenting clinical signs are lethargy, fatigue, and pallor. Unlike subacute IHA, the anemia is unresponsive, almost

always Coomb's antibody negative, and systemic signs such as hepatosplenomegaly, lymphadenopathy and icterus are not apparent. This form of IHA is probably associated with autoantibodies that react with surface antigens present on red cells that are late in differentiation, but not yet released into the circulation. This explains the absence of Coomb's antibody positivity and spherocytosis. An indirect Coomb's test is sometimes positive when the animal's red cells are treated with bromelin, ficin or trypsin and then incubated with its serum. Such treatment may expose more deeply situated antigens not present on circulating mature red cells but that were arrayed on the surface at an earlier stage of differentiation. As expected, the bone marrow shows either a hyper- or normal-responsive erythroid series. Multiple incompatibilities are sometimes seen on cross-match even though the animal may never have been transfused before. Chronic IHA has to be differentiated from congenital red blood cell enzyme deficiencies (heritable spherocytosis) associated with erythrocyte phosphofructokinase (American Cocker spaniel; English Springer spaniel) and erythrocyte pyruvate kinase (Basenji) deficiencies (Giger and Noble, 1991; Giger et al., 1992).

Chronic IHA is treated initially with prednisolone, and if there is no response in 14–21 days, cyclophosphamide, azathioprine or leflunomide is added to the regimen. Blood transfusions may be necessary to sustain animal until therapy takes effect.

3.2.1.4. Cold agglutinin disease. Cold agglutinin disease is rare type of IHA, which is associated with autoantibodies that bind to the red cell surface at lower than core body temperatures. Cold agglutinins are usually of the IgM class and are often idiopathic in origin. Clinical manifestations of disease are usually associated with cold exposure; therefore, dogs with cold agglutinins are most apt to be seen for the condition during colder weather and in colder regions. Two types of disease signs may occur: hemolytic anemia; and/or gangrene of distal extremities. Hemolytic anemia results from the binding of cold agglutinins in the cooler peripheral capillaries; although the antibodies detach when the cells return to the core of the body, complement remains bound. Gangrene of distal extremities results from the agglutination of red cells in smaller vessels.

3.2.1.5. Pure red cell aplasia. Pure red cell aplasia of humans occurs in two forms: (1) a congenital form; and (2) an adult acquired form. The congenital form in humans is known as Diamond–Blackfan syndrome (Dianzani et al., 1996). The syndrome is definitely familial in 10% of patients and sporadic in the remainder. The adult form of pure red cell aplasia has been associated with granular lymphocyte-proliferative disorders (Yamada et al., 1997), thymoma (Masuda et al., 1997), drugs (Thompson and Gales, 1996), parvovirus B19 infection, other immune diseases (Tsai et al., 1997), development of antibodies to either recombinantly derived or natural endogenous erythropoietin (Prabhakar and Muhlfelder, 1997), or idiopathic.

Pure red blood cell aplasia in dogs is similar to the human disease; congenital and idiopathic etiologies have been observed. A congenital pure red cell aplasia occurs in puppies from 2 to 4 months of age. Clinical signs include fatigue and extreme pallor, and in very young animals, growth may be retarded. The anemia is totally unresponsive, while WBC and platelet counts are normal. The Coomb's test is negative, spherocytes are absent, and like chronic AIHA, compatible donors are sometimes difficult to find. The

reason for this higher than normal incidence of allo-reacting antibodies is unknown. The diagnostic tip-off is the existence of a marrow aplasia that involves only one cell-line. The aplasia may be total if the primitive RBC precursor cells are the target or there may be a maturation arrest at some later stage if more differentiated cells are the target. This specificity indicates immune-mediated disease rather than toxins, myeloproliferative disorders, etc.

The treatment is identical as that for subacute or chronic IHA, except that more time must be allowed for the stem cells to be regenerated and the anemia to began to respond. Combination therapy, usually glucocorticoids and cyclophosphamide, will bring about remission faster than glucocorticoids alone. Affected puppies often need to be transfused while waiting for therapy to take effect. The prognosis is good and recurrence is not usually seen.

Adult onset pure red cell aplasia is identical to the congenital form in virtually all aspects except age at onset. About one-third of dogs given human recombinant erythropoietin will also develop anti-erthropoietin antibodies and severe pure red cell aplasia within one month or more of treatment.

3.2.2. Immune-mediated thrombocytopenia (ITP)

Immune-mediated thrombocytopenia is one of the most common immunologic disorders in dogs (Feldman et al., 1988). There is a breed predilection towards poodles, and it has been associated with estrus in intact female dogs. ITP in dogs is idiopathic in 75–90% of cases, but like IHA, it can be secondary to drugs (Handagama and Feldman, 1986, 1988; Sullivan et al., 1992), vaccines (Elliott, 1997), *Ehrlichia platys* and *E. canis* infections (Harrus et al., 1997; Waner et al., 1995), neoplasia (Grindem et al., 1994), or to other immune diseases such as SLE or AIHA. An idiopathic asymptomatic thrombocytopenia has been described in Cavalier King Charles spaniels (Smedile et al., 1997).

Anti-platelet antibodies in the serum of some affected dogs were found to react with membrane antigens GP IIb and/or IIIa, similar to the situation in humans (Lewis and Meyers, 1996). Platelet antibodies in serum or eluted from platelet membranes were found to react with homologous platelets in 58% of the cases (Lewis and Meyers, 1996).

The most frequent presenting clinical signs are petechial and ecchymotic hemorrhages of the skin and mucous membranes. Hemorrhages are often noticed during or after clipping. Epistaxis, melena, hematuria, hyphema, and even neurological signs, may be seen in severe cases. There can be substantial blood loss and anemia in dogs with severe ITP, and it may be difficult to determine whether the primary problem is ITP with secondary hemorrhage, concurrent ITP and IMHA, or blood loss from some other source and DIC.

The critical laboratory finding is a thrombocytopenia, usually below 60 000 platelets/ ul. Anemia, often responsive, accompanies cases that have either IHA or excessive blood loss. Platelets in the blood are often large and bizarre shaped. Megakaryocytes in bone marrow are normal in numbers or increased in 90% or more of cases. Although megakaryocytes may be present in normal or increased numbers, they often appear immature, fragmented, or fragile. Megakaryocytes are severely decreased or even absent in <10% of cases; such a finding is suggestive of a 'pure megakaryocytic aplasia'.

Clotting tests, including PT, PTT, ACT, and FDPs are normal. Anti-platelet antibodies, as determined by platelet factor-3 release test are present in 70% or less of the cases. A fluorescent antibody test for antiplatelet antibodies can be done on bone marrow smears, providing megakaryocytes are present; it is about 40% sensitive (Kristensen et al., 1994a, b). An indirect platelet immunoflourescence test appears to have a higher sensitivity (70%) (Kristensen et al., 1994a, b). Adaptation of the latter test to flow cytometry did not improve the sensitivity. Using sensitive binding tests, platelet bound antibodies were found in over 90% of affected dogs and serum anti-platelet antibodies in 34% (Lewis and Meyers, 1996; Lewis et al., 1995). Because of the relative insensitivities of anti-platelet antibody tests the diagnosis of ITP is usually made on clinical appearance and response to therapy. Care must be taken to rule out secondary causes of ITP or of thrombocytopenia.

There are two main findings that influence the mode of therapy: (1) the presence or absence of megakaryocytes; and (2) the presence and severity of associated blood loss. The absence of megakaryocytes will delay the anticipated therapeutic response for a week or more, which can greatly effect the cost and prognosis in animals with severe ITP and blood loss. In animals with petechial and ecchymotic hemorrhages, but without serious blood loss, glucocorticoid treatment alone will usually suffice. Azathioprine or cyclophosphamide is usually added to the regimen if there is no response in 10–14 days. Thrombocytopenia associated with severe blood loss is best treated with prednisolone and vincristine, because vincristine will frequently decrease the normal response time from 7–10 days down to 3–5 days. Fresh whole blood, collected in such a manner as to preserve platelets, is required in animals that are manifesting severe hemorrhage.

In about 10–20% of the cases, the ITP will be very difficult to keep in remission with safe levels of prednisolone alone, or even with combination prednisolone/cytotoxic drug therapy. In such cases, consider treating with prednisolone and leflunomide, danazol and prednisolone, or drug therapy combined with splenectomy.

A statistical linkage between thrombocytopenia and eventual development of lymphoma has been described in dogs (Keller, 1992). Affected dogs are 5.61 times more likely to develop this tumor than animals in the general population.

3.2.3. Mixed cytopenias

Mixed cytopenias of an immune nature are known in human medicine as Evan's syndrome. In a study of 42 patients, the median age of onset was 7.7 years (range 0.2–26 years); 32/42 had thrombocytopenia, 28/42 had anemia, 10/42 had neutropenia, and 6/42 were pancytopenic (Matthew et al., 1997). Intravenous immunoglobulin and glucocorticoids, the first line of therapy, were variable in effectiveness and but positive responses were sustained for two years. Splenectomies performed on 15 patients were effective for a median of one month. Other treatments included cyclosporine, vincristine, azathioprine, cyclophosphamide and danazol. The course of disease was characterized by recurrent thrombocytopenia, hemolytic anemia and neutropenia. After two years, one-third of the patients were in remission, but two-thirds were still on treatment or had died.

Mixed cytopenias that closely resemble human Evan's syndrome occur frequently in dogs, with predisposition to purebreds and Cocker spaniels. Like the human disease, affected dogs usually manifest because of the anemia, and thrombocytopenia is detected

during the clinical work-up, or vice versa. Many dogs with Evan's syndrome will also be leukopenic, suggesting a broad-based attack on the bone marrow. The various cytopenias may occur simultaneously, manifest variably during the same attack, or appear to one degree or another upon subsequent attacks. Care must be taken not to diagnose Evan's syndrome in dogs with immune- or non-immune-mediated blood loss and consumptive thrombocytopenias. For example, many dogs with peracute IHA will have an associated DIC and consumptive thrombocytopenia. The same is true for dogs with severe babesiosis or haemobartonellosis. Dogs with Evan's syndrome are less likely to respond to glucocorticoid therapy alone, and are more difficult to get into remission with combination immunosuppressive drug therapy, as is the case with the human disease (Matthew et al., 1997). Relapses may occur at varying times after cessation of treatment. Glucocorticoid and cyclosporine therapy is indicated in difficult-to-treat cases in humans (Emilia et al., 1996; Rackoff and Manno, 1994), and has proved useful in dogs. Leflunomide may prove to be of significant benefit to dogs with refractory disease (Gregory et al., 1998).

3.2.4. Immune-mediated neutropenia

Autoimmune neutropenia is a relatively rare disorder. Like other immune cytopenias, it occurs in both idiopathic and secondary forms. Secondary disease is usually associated with drugs, in particular anticonvulsants and antibiotics (Jacobs et al., 1998). It must be differentiated from pre-myeloproliferative disease and heritable cyclic neutropenia.

Autoimmune neutropenia usually presents with acute fever and depression and a profound neutropenia is noted on routine a CBC. Bone marrow cytology is either normal or demonstrates a hypercellular granulocytic series. There are no good tests for autoantibodies to neutrophils. There is usually a good response to long-term prednisolone, or combination immunosuppressive drug treatment. Some dogs may go on to develop myeloid neoplasias.

3.2.5. Alloimmune hemolytic anemia

3.2.5.1. Transfusion reactions. Transfusion reactions are the most common alloimmune disorders of dogs. Dogs have at least 13 different dog erythrocyte antigens (DEA), with eight being internationally standardized (Hale, 1995). Test reagents are available for six of the eight standard canine blood types, DEA 1.1, 1.2, 2, 3, 4, 5 and 7. DEA 1.1 and 1.2 are strongly antigenic in dogs negative for these blood types and the antibodies are very lyric. Antibodies to DEA 3, 5 and 7 are not lyric, but will cause splenic sequestration and subclinical destruction of transfused red cells over a several day period. Antibodies to DEA 4 have no effect on the survival of DEA 4 positive red cells, and for this reason, DEA 4 positive dogs are considered to be the ideal universal donors.

Transfusion of DEA 1.1 and 1.2 positive red cells into dogs sensitized against these antigens is of great clinical significance, while transfusion of DEA 3, 5 and 7 red cells into dogs sensitized to these antigens is only significant in terms of transfusion efficacy. Although the vast majority of severe transfusion reactions involve alloantibodies to DEA 1.1 and 1.2, at least one dog that was DEA 1.1 negative developed highly lyric antibodies to an undetermined blood type following an initial transfusion, and a second transfusion

from a DEA 1.1 negative donor resulted in a serious hemolytic episode (Callan et al., 1995).

Similar to the situation of the human ABO and cat AB blood groups, many dogs that are DEA 3, 5 and 7 negative will have naturally occurring alloantibodies to these blood types. However, given the infrequency of these blood groups and the non-lyric nature of anti-DEA 3, 5 and 7 antibodies, such natural antibodies are not of great clinical significance. The greatest risk is to DEA 1.1 and 1.2 negative dogs that have been previously transfused with blood from DEA 1.1 and 1.2 positive donors; these dogs will develop severe reactions to subsequent transfusions with DEA 1.1 and 1.2 positive blood. Sensitization need not involve previous transfusion; a surprising number of dogs with IHA, and no previous transfusion history, will also possess alloantibodies against a range of red cell surface antigens, and these alloantibodies can be clinically significant. The precise red cell antigens involved in such animals have yet to be determined.

Severe signs of blood incompatibility will usually began to appear within 5–15 min of transfusion. The earliest signs are an increase in pulse and respiration, shivering, fever, and edema of the face (angio-neurotic edema). Subsequent signs may include hemoglobinuria; cardiac arrythmias; pulmonary edema; renal shut-down; shock; DIC; thrombosis of vessels in the feet, tips of tail and ears leading to gangrene; and death.

Recipient and donor dogs must be cross-matched prior to transfusion. The 'major cross-match' involves testing the serum of the recipient against washed RBC of the donor for gross agglutination. An equal volume of a 1 : 5–1 : 10 dilution of the recipients serum in saline is mixed with an equal volume of a 2% suspension of washed donor RBC in saline. Agglutination will appear within 5–60 min if agglutinins are present. Although gross-agglutination tests will prevent most donor/recipient mismatches, the author has observed at least two instances where severe transfusion reactions occurred in the face of negative agglutination tests. In both cases, the recipient was found to contain non-agglutinating antibodies to donor red cells in an indirect Coomb's test. The indirect Coomb's test involves preincubating donor RBCs with recipient serum for an hour or so and washing and resuspending them in saline before adding the Coomb's reagent. Agglutination occurring after the addition of the Coomb's reagent is evidence for the presence of non-agglutinating antibodies in the recipient's serum.

The 'minor cross-match' involves testing the donor's serum against the recipient's RBCs. Agglutination indicates that the serum of the donor may react with the donor's RBCs following transfusion if the plasma component is given along with the RBCs. This reaction has only minor clinical significance, hence the term minor cross-match incompatibility. Nonetheless, minor mismatched transfusions should only be done in situations where a better matched donor is not available.

Blood transfusions in dogs should always be undertaken with care (Harrell and Kristensen, 1995). In order to avoid transfusion reactions, donor and recipient should be cross-matched prior to transfusion. This should be done even when donors are DEA 1.1 and 1.2 negative. The blood should be transfused slowly for the first 5–10 min whenever possible, and if adverse reactions are noted in the first few minutes, the transfusion should be aborted. The worst thing is to have given all of the blood prior to the onset of adverse signs. Pretreatment with antihistamines and corticosteroids will not prevent the reaction from occurring, but may make it somewhat milder. Once a severe transfusion reaction

occurs, treat with massive dosages of steroids, control shock, support renal function, and be prepared to give oxygen. An immune complex vasculitis has been observed following some acute transfusion reactions and can lead to major tissue infarctions 24–72 h after initial signs have passed.

3.2.5.2. Neonatal isoerythrolysis. Neonatal isoerytholysis has been described in puppies, associated mainly with DEA 1.1 (Hale, 1995). A small proportion of DEA 1.1 negative bitches can be sensitized to DEA 1.1 if carrying DEA 1.1 positive puppies, probably by transplacental leakage of fetal red cells. The bitch will then make alloantibodies to DEA 1.1, which will be passed to the puppies in the colostrum. Clinical signs appear within the first day or two after nursing and include anemia, lethargy, jaundice, tachypnea, tachycardia, hepatosplenomegaly and occasionally hemoglobinuria. Mildly affected puppies can be treated symptomatically with rest and good nursing care. Severely affected puppies may require extensive blood transfusion. When in doubt as to which donor to use, washed (plasma-free) RBCs from the bitch is an obvious choice because there is negligible chance that they will be destroyed by the puppy.

3.3. Non-cytolytic diseases involving autoantibodies

3.3.1. Myasthenia gravis

Myasthenia gravis occurs in an acquired (autoimmune) and congenital (familial or genetic) form in both man and dogs. The congenital form does not have an immune basis, while the acquired form is associated with the production of antibodies to acetylcholine receptors. These antibodies lead to a diminution of these structures on the postsynaptic sarcolemma. The acquired form is far more common than the congenital form in dogs, and the incidence of acquired myasthenia gravis in dogs is even greater than for humans.

Similar to the human disease, myasthenia gravis in dogs has been observed as a sequelae of thymoma. However, this relationship is not nearly as common in dogs as in people (Lainesse et al., 1996; Rusbridge et al., 1996). In a study of 23 canine thymomas, 11 of the animals had megaesophagus, and myasthenia gravis was confirmed in seven of these 11 (Atwater et al., 1994). Paradoxically, one dog developed clinical signs of myasthenia gravis after removal of the thymoma. None of the dogs had evidence of distant metastasis. Histologically, the predominant tumor types were differentiated-epithelial (9/23) and lymphocyte-rich (6/23).

Shelton et al. (1997) studied 1154 dogs residing within the United States from 1991 to 1995 with acquired myasthenia gravis. Purebred dogs had a greater risk than mixed-breeds; dogs with the highest risk of acquired myasthenia gravis were Akitas, terrier group, Scottish Terriers, German Shorthaired Pointers, and Chihuahuas. Rottweilers, Doberman Pinschers, Dalmatians, and Jack Russell Terriers had low relative risks. Sexually intact males and dogs less than one year old had some protection from risk.

Several clinical forms of acquired myasthenia gravis have been described (Dewey et al., 1997; Webb et al., 1997). About one-third of affected dogs had no historical or clinical evidence of appendicular muscle weakness, and are designated as focal myasthenics. Dogs with focal myasthenia usually exhibited weakness in facial, pharyngeal, and laryngeal muscles.

About two-thirds of myasthenic dogs present for systemic disease, usually combinations of appendicular weakness and megaesophagus. The systemic form of myasthenia in dogs is subdivided into chronic generalized and acute fulminating forms; one-fourth of dogs have a fulminating onset and three-fourths chronic. Most of the dogs with systemic myasthenia, regardless of form, will manifest signs of megaesophagus, and a smaller proportion will demonstrate facial, laryngeal, or pharyngeal weakness. Historical evidence of exercise-associated appendicular weakness is observed in about one-half of dogs with chronic generalized myasthenia gravis, while dogs with acute fulminating myasthenia gravis usually presented with sudden weakness. About one-half of dogs with either chronic or fulminate generalized myasthenia demonstrate primarily pelvic limb weakness.

Third degree atrioventricular block was diagnosed in four dogs with acquired myasthenia gravis and megaesophagus (Hackett et al., 1995). Two of these dogs also had mediastinal thymomas. Three dogs in a series of 23 with thymomas also developed third-degree atrioventricular heart block, one of which had generalized myositis involving the cardiac muscle (Atwater et al., 1994). Therefore, heart problems may complicate myasthenia gravis in dogs.

The diagnosis of myasthenia gravis is based on signalment (i.e., breed, age), history, clinical findings, results of IV edrophonium chloride administration, electromyographic findings (decremental reduction of amplitude on repetitive nerve stimulation), presence or absence of muscle membrane staining by immunocytochemical methods, serum acetylcholine receptor antibody concentration (Holland et al., 1994), treatment, and outcome. An acetylcholine receptor antibody titer of >0.6 nmol/l is considered diagnostic for acquired myasthenia gravis (Shelton et al., 1997).

One half of myasthenic dogs die or are euthanatized shortly after admission to the hospital due to aspiration pneumonia. The dogs with acute fulminating myasthenia gravis had a markedly higher one-year mortality rate in comparison with the other two groups. The principal treatment is anti-cholinergics such as pyridostigmine bromide, which will usually improve overall muscle strength. The additional use of immunosuppressive therapy had a significant positive effect on patient survival, regardless of the type of myasthenia gravis. Surgical removal of thymomas in dogs with these tumors has had mixed results. In two German Shepherd Dogs, the disease did not resolve, but there was a more satisfactory control of clinical signs with anticholinesterase treatment (Rusbridge et al., 1996) Surgical removal of a thymic mass in an older Cocker Spaniel resulted in rapid resolution of clinical signs and a decrease in serum acetylcholine–receptor antibody concentration, but signs recurred six months postoperatively with regrowth of the thymoma (Lainesse et al., 1996). Two dogs with thymomas, megaesophagus, and third degree heart blocks died of aspiration pneumonia within three months of surgical resection of their tumors (Hackett et al., 1995). Equally poor results were obtained in a larger study; six of nine dogs with thymoma and megaesophagus that underwent surgery died or were euthanatized within one week of diagnosis (Atwater et al., 1994).

3.3.2. Tempormandibular myopathies (Masticatory myopathies)

Dogs appear to be uniquely susceptible to autoimmune disorders involving the masticatory muscles (Lewis, 1994; Shelton and Cardinet, 1987). Purebreds are

predisposed to the condition, and the Cocker spaniel may be over represented. The specificity of these diseases is probably related to the unique structure of these muscles in dogs. The masticatory muscles of dogs have a unique myofiber proteins (myosin enzymes and myosin light and heavy chains) compared to limb muscles (Shelton et al., 1985a, b, 1988). Autoantibodies against unique type 2 M fiber proteins can be found bound in the muscle and free in the serum (Shelton et al., 1987). Antibody targets included a 185 kDa protein, myosin heavy chain, and light chain-2 masticatory myosin.

Masticatory myopathies may be manifested acutely with swelling of the tempomandibular muscles and severe pain on opening of the mouth. In many dogs, however, this acute stage goes unnoticed and the muscle disease is more chronic and low-grade; the muscles slowly atrophy and are replaced by fibrous tissue. There is no obvious pain and the first clinical abnormality is often manifested in difficulty in eating and drinking associated with an inability to open the mouth as a result of muscle and tendon contracture. Eosinophilia may or may not be present. The diagnosis is usually based on the characteristic clinical signs and muscle biopsy. Antibodies can be measured in the serum against normal masticatory muscle proteins.

Affected dogs are treated initially with high doses of prednisolone. The jaw slowly begins to open after several weeks on treatment, although usually never back to normal. The treatment is discontinued, therefore, after the animal can open its mouth enough to eat and drink comfortably.

3.3.3. Bullous dermatitides

The bullous dermatitides are characterized by the formation of autoantibodies to various intracellular cement proteins of mucocutaneous junctions and/or skin. The binding of antibodies to intracellular cement leads to the breakdown of the bonds between various cell layers leading to a cleft, which is then filled with fluid and forms a blister or bullae. The blister rapidly ruptures leaving an underlying ulcer that varies in depth according to the layer of the epidermis that is affected. The layer of the epidermis that is affected is, in turn, dependent on the particular intracellular cement substance that is the target of attack. Lesions tend to be concentrated on mucocutaneous junctions of the mouth, eyes, anus, vulva, prepuce and skin in certain areas of the body (depending on the particular disease).

3.3.3.1. Pemphigus foliaceous

. Pemphigus foliaceous is surprisingly common in dogs and has been associated with the formation of autoantibodies to epidermal proteins of 85, 125, and 160 kDa (Iwasaki et al., 1996, 1997). The 160 kDa protein is of the same size as human desmoglein 1, the predominant antigenic target in pemphigus foliaceous in people. The disease occurs predominantly in purebreds, with a large number of breeds represented (Kuhl et al., 1994). However, Akitas, English Springer Spaniels, Chow Chows, Chinese shar pei and Collies are from 4 to 40 times more likely to develop pemphigus foliaceous than other breeds.

Pemphigus foliaceous in dogs usually occurs between the ages of two and seven years and is gradually (75% of cases) or rapidly progressive (25% of cases). This is the most superficial of the bullous dermatitides. The lesions are generalized, patchy, facial (80% of cases), or pedal orientated. The lesions in 50% of the dog appear first over the bridge of

the nose. Lesions are characterized by crusting, scaling, and alopecia. The inside of the mouth is usually unaffected.

The disease in Akitas, and less often in other breeds, may be associated with uveitis. Such cases may be mistaken for the Voight/Koronada/Harada (VKH) syndrome, which occurs most commonly in people of Japanese extraction. VKH is due to a cell-mediated autoimmune attack on melanocytes, which in humans is often manifested by uveitis, meningitis, and depigmenting skin lesions. A VKH-like syndrome does occur in dogs, but mainly in Chow chows.

Biopsies of affected skin of dogs with pemphigus foliaceous shows vesicopustules directly beneath the stratum corneum (subcorneal pustules). Microabscesses may also be observed within the external root sheath or within hair follicles. The pustules are filled with acantholytic keratinocytes, neutrophils, and eosinophils. Indirect fluorescent and immunoperoxidase antibody staining shows the subcorneal deposition of immunoglobulin in areas of intracellular cement (Shinya et al., 1996). Sera from 65% of affected dogs will have antibodies that react with bovine esophagus. Bovine esophagus is the substrate of choice when compared to bovine nose or tongue, monkey esophagus and canine nose (Iwasaki et al., 1996).

About 40% of animals will respond to high dose steroid treatment followed by maintenance treatment. Drug-free remission is very rare. Animals that fail to respond well to glucocorticoids alone, or which require toxic levels to keep the disease under control, can be treated with glucocorticoids and azathioprine, glucocorticoids and cyclophosphamide, or glucocorticoids and gold salts. Drug toxicities become a problem with time, because of the difficulty of achieving full remission or of sustaining remission once treatment is discontinued. Leflunomide and a glucocorticoid used in combination may provide better long-term control and less side-effects.

3.3.3.2. Pemphigus vulgaris. Pemphigus vulgaris is a much less common condition than pemphigus foliaceous and predominates in purebreds. Unlike pemphigus foliaceous, which tends to spare the mucocutaneous junctions, pemphigus vulgaris has a predilection for the mucocutaneous junctions of the mouth, eyes, nostrils, anus, prepuce, rectum or nipples and the mucosa of the inside of the mouth (cheeks, palate, tongue). Involvement of the nail beds is also seen. The lesions are frequently secondarily infected with bacteria and are painful and pruritic. Affected animals are often systemically ill, as demonstrated by malaise and weight loss.

Intact vesicles can sometimes be seen upon frequent and careful examination of the mucous membranes. These blisters are due to a subrabasiler cleft formed around acantholytic keratinocytes. Signs of secondary bacterial infection and inflammation are common.

The same as for pemphigus foliaceous, but may need to treat for secondary bacterial infection during the initial stages of therapy. However, remission is more difficult to achieve and the long-term prognosis is even worse.

3.3.3.3. Bullous pemphigoid. Bullous pemphigoid is uncommon among all breeds of dogs, but Shetland sheepdogs, Collies, and perhaps Doberman pinschers, may be over-represented. Dogs are usually affected as young adults. Lesions appear as deep ulcers of

the skin and mucocutaneous junctions, often having an outward appearance similar to full-thickness burns. Lesions are concentrated on mucocutaneous junctions of the oral cavity, anus, vulva or prepuce, and skin of the axilla and groin. Bullae may be seen upon careful examination. The bulla involves separation of the entire epidermis from the dermis along the basal lamina. Indirect fluorescent antibody staining demonstrates binding of IgG to the basal lamina. Autoantibodies in one dog were found to react with a 180 Kd glycoprotein from canine skin analogous to the 230/180 Kd bullous pemphigoid (BP) antigen of humans (Iwasaki et al., 1995a).

The condition carries a poor prognosis due to difficulty in achieving and maintaining full remission. The treatment of choice is a glucocorticoid used in combination with azathioprine. Combined glucocorticoid/leflunomide therapy needs to be evaluated for this condition.

4. Immune complex diseases (Type III reactions)

4.1. Immunopathogenesis of immune complex disorders

Immune complex diseases involve:

1. formation of circulating antigen–antibody complexes;
2. passage of immune complexes through endothelial gaps;
3. complement fixation;
4. inflammatory response; and finally,
5. localized vascular damage.

Therefore, the hallmark lesion of immune complex disease is vasculitis. The vasculitis can be associated with acute inflammation (e.g., synovitis, uveitis), vascular thrombosis and tissue necrosis (e.g., glossal and palatine ulcers), hemorrhage (e.g., purpura hemorrhagica), or a more insidious disease of the vascular wall (e.g., glomerulonephritis).

Immune complexes (antigen–antibody complement aggregates) are generated in situations where both antigens and corresponding antibodies coexist in the blood and/or tissues. Chronic antigen formation occurs in the following situations:

1. infectious diseases;
2. drugs;
3. acute or chronic use of foreign proteins; and
4. autoimmune diseases.

All microbial infections generate immune complexes. In the case of acute diseases such as influenza, the complexes exist only for the period of time that the organisms are actively replicating. During this time, however, they may contribute to the overall clinical syndrome, e.g. transient muscle and joint pain, fever, etc. Persistent immune-complex related signs may occur, however, if the infection should become chronic.

Four types of interactions between the drug and tissue can lead to antibodies: the drug binds directly to a tissue protein, a drug-carrier complex is formed, and antibodies are

directed against the drug (hapten) moiety; the drug is biotransformed into a more haptenogenic compound, which then binds with tissue proteins and elicits antibodies to the biotransformant; the drug or its metabolite interacts with the tissues and forms a new antigenic site; or the drug or its metabolite reacts with the tissue and causes conformational changes in the tissue protein rendering it antigenic.

Serum sickness is a classical disease resulting from the use of heterologous antiserum. Immune serum is used for the treatment or prevention of certain infections or toxicities. After the initial inoculation of foreign protein, antibodies appear in three to seven days. Immune complexes are then formed and clinical signs of fever, lymphadenopathy, rash, and joint and muscle pain appear. These signs will persist until all of the foreign protein is bound and the complexes are removed by the RE system.

Certain immune complex diseases involve antibodies to self-antigens. Immune complexes in SLE consist of antinuclear antibodies and corresponding nuclear antigens. In rheumatoid arthritis (RA) of man, and possibly RA of dogs, autoantibodies are produced against denatured or immune complexed IgG. These autoantibodies are called rheumatoid factors. Rheumatoid factors also appear in the blood of some animals with chronic infections. Rheumatoid factors are one way that the body has of clearing smaller-sized immune complexes. Rheumatoid factors are only pathogenic when their production is excessive. In such cases, they may contribute to immune complex disease, as they do in rheumatoid arthritis of people.

Immune complexes of large size (created in the zone of antibody excess) are cleared directly by the RE system. Small complexes (created in the zone of antigen excess) are very small and move freely between the intra- and extra-cellular fluid spaces. Immune complexes generated under the conditions of slight antigen excess, however, are intermediate in size and are more likely to be deposited in the endothelium. Immune complexes of proper configuration will pass through the endothelial gaps but will become embedded within the basement membranes. Immune complexes may also form in situ in the area of the basement membranes rather than be carried in a preformed fashion from other sites. This will occur in situations where the proteins of the basement membranes are themselves immunogenic, or in situations where free antigen is trapped in the basement membrane and antibodies to the antigen are generated at a later time.

The likelihood of immune complexes depositing in a given organ depends on several factors:

1. the physical or anatomic make-up of the endothelial bed;
2. relative blood flow to the organ;
3. conditions increasing vascular permeability, i.e. histamine release;
4. the specific chemical make-up and charge of the complex; and
5. genetic factors.

Immune complexes are in equilibrium with free antigen and antibodies in the tissues. The rate of complex deposition will vary therefore with the amount of antigen and antibody formed at any given period of time. Immune complex formation is a normal reaction to most acute infectious or antigen-releasing disorders. Most complexes that are deposited acutely in basement membranes are rapidly dissolved when the period of antibody excess

(convalescence) is reached, which occurs before there is any permanent damage to the basement membranes.

The deposition of immune complexes per se does not lead to disease. Problems arise only when the host responds to the complexes and a local inflammatory reaction is elicited. Immune complexes were initially thought to cause localized inflammation by binding complement and attracting neutrophils into the area; inflammatory mediators released by the neutrophils were ultimately responsible for the lesions. Immune complex lesions, however, can also occur in the absence of neutrophils or complement. The role of macrophages, and macrophage kinins, in immune-complex induced inflammation is currently an important area for study. Macrophages are one of the most consistent inflammatory cells found in immune complex lesions.

4.2. Specific disorders involving immune complex-induced vasculitis

4.2.1. Systemic lupus erythematosus (SLE)

Any discussion of SLE in dogs should be prefaced by a description of the disease in people. Lupus or 'wolf-like' refers to the patchy depigmentation and erythema of the skin on the face of humans with SLE. However, skin lesions are only one part of the syndrome. In fact, virtually every organ system in the body can be affected in people with SLE.

SLE is a common disorder, seen in 1 : 2000 humans. It affects females more than males (4 : 1), and blacks more than whites. Most patients are 10–50 years of age when the first signs are seen, with a particularly high incidence in the 20–40 year age group. There is some familial clustering in people. Offspring of affected individuals have a twofold or more higher incidence, and relatives of people with clinical SLE often have serological evidence of the disease but no clinical signs. Humans with SLE are more likely to have the HLA-DR2 or -DR3 leukocyte types than non-affected individuals. DLA-A7 was found to have a strong positive relationship to SLE in German shepherd dogs, while DLA-A1 and DLA-B5 were negatively related (Teichner et al., 1990).

The disease in man runs several courses.

1. Fulminating disease with a rapid progression of signs.
2. Cyclic disease in which the first attacks are the most severe and frequent; over a period of 5–10 years the attacks become milder and further apart.
3. Chronic low-grade disease, occasionally interrupted by periods of increased disease activity.
4. Serologically positive individuals with few if any signs of illness.

Any number of organ systems can be involved in SLE. There is a tendency, however, for the disease to localize in one or two organs. The most life-threatening complication of SLE in man is kidney failure, followed in frequency by neurological complications. The 10-year survival rate of people with SLE now exceeds 85%.

SLE has been recognized in almost all species of animals of veterinary importance. It has been best described, however, in the dog. Canine SLE is as prevalent, or perhaps more so, than its human counterpart. It occurs mainly in purebred, especially sporting breeds

such as Spaniels, Pointers, Retrievers, German Shepherd dogs, and Doberman Pinschers. It is also common in many non-sporting breeds such as the Spitz and Poodle. The usual age of onset in dogs is from 1 to 8 years of age, with most cases occurring in the 2–4 year range. This is similar to the proportionate age incidence for people.

SLE is often diagnosed on the basis of the presence of certain typical symptoms and the absence of exclusionary diseases, and attempts have been made to utilize such criteria for canine SLE. However, if human criterium are strictly adhered to, most dogs with 'SLE' would not qualify. Dogs tend to have one or two systemic signs, often polyarthritis, while skin, hematologic, CNS, and renal manifestations are less important (Pedersen et al., 1976a; Krum et al., 1977; Monier et al., 1978; Slappendel et al., 1972).

The etiology of SLE in man and animals has not been determined, although gender and genetics play a role. Estrogen enhances disease, while testosterone is protective. Close relatives of SLE patients are 2–5 times more likely to develop SLE. In man, almost 25 different drugs, and in particular hydralazine and procainamide, will also induce a lupus-like syndrome. Drug induced disease differs, however, from naturally occurring SLE in the following ways:

1. the sex ratio is equal;
2. nephritis and CNS signs are not seen;
3. serum complement levels are usually normal and antibodies to native DNA are absent;
4. clinical and laboratory abnormalities return to normal when the drugs are discontinued.

SLE has been seen in 20% or less of aged cats chronically treated with prophylthiouracil for hyperthyroidism, but drug induced SLE has not been described in dogs.

SLE in man and animals has many features in common, but the hallmark is antinuclear antibody (ANA). ANAs seem to be produced in response to a slow and insidious release of nucleic acids in the presence of a hyper-responsive immune system. Once formed, ANAs will react with this slowly released nucleic acid and form immune complexes. The antinuclear antibodies are directed against single stranded or denatured DNA (ssDNA), saline soluble (extractable) non-DNA nuclear antigens, Sm (Smith) nuclear antigen, histones, and ribonucleoprotein (Goudswaard et al., 1993; Monestier et al., 1995; Thoren-Tolling and Ryden, 1991; White et al., 1992). Antibodies to nuclear glycoproteins not associated with DNA have also been detected in dogs with SLE (Soulard et al., 1991). Antibodies to double stranded DNA, a hallmark of human SLE, may not be as important in dogs (Jones, 1993).

Unlike the situation seen in type II immune diseases, anti-nuclear antibodies are not in themselves pathogenic. Mothers with SLE can pass on high titers of ANA to their babies, but there is no evidence of disease in the infants. Furthermore, SLE in people and animals sometimes occurs in the absence of detectable ANA (seronegative lupus). By definition, such individuals do not have SLE, because ANA is a diagnostic criteria. The disease syndrome in ANA negative individuals is otherwise indistinguishable.

SLE has been called the prototypic immune complex disease; circulating immune complexes pass through endothelial cell junctions and are trapped in the underlying basement membranes. Complement is bound to the complexes, and this in turn can lead

to the influx of polymorphonuclear cells and localized inflammation. This reaction is localized around blood vessels, hence vasculitis is one of the hallmarks of SLE. If the vascular reaction occurs in glomeruli, glomerulonephritis is the result. Vasculitis in synovial blood vessels results in synovitis; meningeal vessels-meningitis; retinal vessels-uveitis; serosal vessels-peritonitis or pleuritis; spinal cord vessels-transverse myelopathies; cardiac vessels-myocarditis; pericardial blood vessels-pancreatitis; GI tract-colic and ulcers, etc. Immune complexes in the skin are usually found around small dermal vessels and in the basal lamina. Deposition of immune complexes in the skin does not always cause lesions by itself, but is potentiated by exposure to solar radiation. Lesions on the extremities (face, hands, ears, bridge of nose, etc.) are therefore, the most striking.

People and animals with SLE suffer from several immunologic abnormalities besides immune complex disease. SLE is associated with a state of immunologic hyper responsiveness, mainly in the B-cell system. There is a deficiency of T-suppressor lymphocytes and an increase in antibody levels to many common antigens, especially of viral origin. Individuals with SLE also have a greater than normal propensity to make autoantibodies, especially to RBCs, platelets, and leukocyte antigens. Antibodies to coagulation proteins (circulating anticoagulants) develop in 10–15% of humans with SLE and have been reported in a dog (Stone et al., 1994). Anti-coagulants are against phospholipids and intrinsic clotting factors such as factor VIII. These antibodies can cause thrombotic or hemorrhagic complications in some people and dogs. Humans with SLE also frequently develop false positive serologic tests for syphilis. This is due to the development of cross-reacting antibodies to phospholipids that are present in many organs and on the syphilis spirochete. Some dogs with SLE will produce antibodies to heat shock proteins (Bell et al., 1995). About 30% of the humans with SLE develop rheumatoid factors, as do a similar proportion of dogs with SLE (Chabanne et al., 1993). Rheumatoid factors are antibodies (IgM) that react with immune complexed or denatured IgG. Lymphocytotoxic antibodies are also detected in the serum of some human and canine patients.

The most common feature of SLE in dogs is a cyclic, antibiotic unresponsive fever accompanied by one or more organ specific signs. Polyarthritis occurs in 70–90% of people and animals with SLE. It is a non-erosive, inflammatory arthritis. Proximal joints are less severely involved than distal joints. Most animals show generalized stiffness, difficulty in getting up and laying down, or a shifting limb lameness. Polymyositis and polyneuritis may also occur in dogs; it is seldom seen by itself and usually accompanies polyarthritis (Krum, 1997). Pleuritis is frequently seen radiographically in dogs with florid SLE, but is usually not clinically apparent. Skin lesions are uncommon accompanying features in dogs compared to humans. Macular erythematous, crusty, and sometimes ulcerative lesions. Usually on the face (bridge of nose, eyebrows, ears, margins of lips). Dermatitis, when seen, is often the predominant feature. Ulcerative (infarctive) lesions of the palate, tongue and digits can be an accompanying or primary feature of SLE in dogs. Neurologic signs are seen in 10–50% of humans, but are uncommon in dogs. Transverse myelopathy is an uncommon sequelae of SLE in dogs and people. Leukopenia, a common sign of SLE in man, is uncommon in dogs. Glomerulonephritis, as a histologic or immunohistologic diagnosis, is common in dogs with SLE, but is much less likely to progress to renal failure than in people.

About 10% or more of dogs with presenting for IHA or ITP have serologic evidence of SLE. Conversely, only a small portion of dogs with the immune complex (i.e., vasculitis) manifestations of SLE will have concurrent IHA or ITP.

The diagnosis of SLE is based on signalment (breed predispositions, age), disease signs compatible with systemic vasculitis and/or autoimmune cytopenias, and ANA positivity. The fluorescent ANA (FANA) test is the standard screening test for all types of ANAs. The FANA is an indirect immunofluorescence assay where serial dilutions of patient serum are reacted with acetone fixed cell culture monolayers, usually human or mouse hepatoma cells, or with rat liver sections (McVey and Shuman, 1991; Thoren-Tolling and Ryden, 1991). FANA titers in animals are usually low (1 : 16–1 : 256) compared to man. Some normal animals will have ANA, so a positive ANA is diagnostic only when it occurs with appropriate clinical manifestations of SLE. The original lupus erythematosus (LE) cell procedure has many false positives and negatives in dogs and is seldom used.

SLE in dogs is treated in a similar manner to SLE in people. The initial attacks of disease are usually the worst and must be treated the most vigorously. Initial treatment often consists of glucocorticoids alone, although combination immunosuppressive therapy is ultimately required in many affected dogs. Once the disease is brought into remission, it can be kept that way with a minimum of drug therapy. About one-half of the animals may be withdrawn from therapy after six months to a year, while the remainder must be treated indefinitely. Glomerulonephritis, when present, is the most difficult part of the syndrome to treat and the disease manifestation that is most apt to be clinically silent until substantial organ damage has occurred. Therefore, renal function should be periodically monitored even through other signs of SLE appear under control. ANA will disappear when animals are in full remission and reappear upon relapse and is therefore a good monitor of disease control.

4.2.2. Discoid lupus

Discoid lupus is the most common disease of the nasal planum of dogs, especially in regions of high solar radiation (White, 1994). The disease occurs most commonly in purebreds with long nose confirmation, but especially in Collies and Shetland sheepdogs. The disease is considered to be a focal form of SLE, with deposition of immune complexes in the basement membrane. Nuclear antigens have been extracted from the lesions of at least one dog with discoid lupus, suggesting that they help make up the immune complexes (White et al., 1992). Disease of the basement membrane renders the skin more susceptible to the effects of solar radiation, which are focused most intensely on the nasal planum and on to the nose. The result is a low grade inflammation of the nasal planum with depigmentation. Constant licking of the lesions can lead to additional damage to the nose. Increased numbers of epidermal langerhan cells expressing MHC class II have been seen in dogs with discoid lupus, but not with pemphigus foliaceous (Day, 1996a, b, c). Antinuclear antibodies are usually not found in the circulation, but have been observed on occasion (Iwasaki et al., 1995b). Treatment is with immunosuppressive drugs, usually Glucocorticoids, sun screens, and avoidance of sunlight. A combination of tetracycline and niacinamide has also shown promise in dog with discoid lupus (White et al., 1992).

4.2.3. Vasculitis occurring secondary to drugs, chronic infectious diseases, autoimmune disorders

Deposition of antigen–antibody complexes and complement in blood vessel walls can cause varying degrees of inflammation, hemorrhage, thrombosis and necrosis in associated tissues. Most forms of vasculitis have a significant dermal component, although lesions may be seen simultaneously in a variety of additional organs. There are several predisposing causes, including:

1. drugs;
2. chronic infectious diseases; and
3. autoimmune diseases such as SLE.

Drug induced vasculitides have been well documented in dogs (Noli et al., 1995). Sulfa drugs, including trimethoprim-sulfa, erythromycin, and lincomycin are frequent offenders in dogs. Gold salts, barbiturates, phenylbutazone, indomethacin, phenytoin, ibuprofen, thiazides, quinine, penicillin and synthetic penicillins, aspirin, procainamide, phenothiazines, anti-thyroid drugs (prophylthiouracil, carbimazole), and a number of other drugs have been commonly associated with this syndrome in people, so the potential to see drug induced vasculitis in dogs will undoubtedly increase the sophistication of veterinary medicine increases and animals are treated with a wider range of drugs and for longer periods of time.

Most drug reactions consist of two components; some type of dermal eruption, with or without systemic signs of fever, and arthralgia. Drug eruptions in people (dermatitis medicamentosa) have been classified into the following categories:

1. toxic erythema (rash);
2. erythema multiform (bullous lesions, frequently bloody);
3. erythema nodosum (patchy erythematous lesions);
4. allergic vasculitis (lesions range from urticaria to necrotic ulcers);
5. purpura (petechial and ecchymotic hemorrhages of the skin);
6. eczema;
7. exfoliative dermatitis (entire skin surface red and scaly);
8. photosensitivity (skin lesions potentiated by sunlight and lesions are more frequent on exposed areas of skin);
9. drug-related SLE;
10. lichenoid eruptions (flat-topped papules, often with scaling and dermal changes);
11. fixed eruptions (erythematous plaques that occur at the same spot each time the drug is reinstituted);
12. toxic epidermal necrolysis (an uncommon condition with severe consequences in some cases, large areas of skin became devascularized);
13. urticaria;
14. pruritus (itchy skin without obvious lesions);
15. hair loss; and
16. pigmentary changes in the skin.

Virtually all of these forms have been seen in dogs to varying degree.

Of these numerous disorders, erythema multiform, allergic vasculitis, drug-related SLE, and toxic epidermal necrolysis are most likely to be caused by immune complex mediated vascular damage. The characteristic clinical feature of these conditions is the abrupt appearance of skin lesions characterized by focal hemorrhage and inflammation, tissue death, and tissue sloughing. Toxic epidermal necrolysis is the most severe of these diseases, with large segments of skin becoming devitalized and gangrenous. Histopathologic lesions are extremely varied and the vasculitis component is sometimes difficult to appreciate. Diagnosis is made on the basis of the appearance of lesions and temporal relationship to drug therapy. The diagnosis is confirmed by response to drug withdrawal.

Vasculitis in one form or another is a common feature of many chronic infectious diseases. Glomerulonephritis, uveitis, polyarthritis, and dermatopathies have been described in a wide range of infections such as monocytic and granulocytic ehrlichiosis, Rocky Mountain spotted fever, Lyme borreliosis, dirofilariasis, pyometra, infectious canine hepatitis, deep mycotic infections, and visceral leishmaniasis.

Vasculitis may accompany other immune diseases in dogs such as SLE, rheumatoid arthritis and hypothyroidism.

4.2.4. Immune vasculitis with no apparent cause (Idiopathic vasculitis)

As the name implies, animals with idiopathic vasculitis have no identifiable predisposing cause. Human diseases of this type include leukocytoclastic angiitis, polyarteritis nodosa, giant cell arteritis, allergic granulomatosis, and Wegener's granulomatosis. Conditions similar to all of these diseases have been seen in dogs. Whether the diseases in animals are exact analogues, however, remains to be proven.

Idiopathic vasculitis in dogs is usually manifested as ulcerative lesions on the palate, tongue, lower limbs, and digits. A systemic vasculitis, with widespread petechia and ecchymoses and subcutaneous hemorrhaging has been observed in Italian Greyhounds; the condition resembles purpura hemorrhagica of man. An idiopathic cutaneous vasculitis with necotizing glomerulitis has been described in racing Greyhounds (Carpenter et al., 1988; Cowan et al., 1997). Systemic recortizing vasculitis has been reported in nine young beagles (Scott-Moncrieff et al., 1992). Acute or chronic edema, especially of one or both hind limbs may also be an unusual manifestation of idiopathic vasculitis in dogs.

The diagnosis of idiopathic vasculitis is made by confirming the presence of vasculitis on deep biopsies and the ruling out of all possible secondary causes. The treatment is similar to other immune diseases, starting with glucocorticoids alone and adding in other immunosuppressive agents if necessary.

4.2.5. Non-erosive immune-mediated arthritides

A non-erosive, non-infectious arthritis occurs in the dog, and though etiologically diverse, it is probably mediated by similar immunopathologic mechanisms (Pedersen et al., 1976b; Pederson and Pool, 1978). The presenting clinical signs of this type of arthritis are similar, whether it is idiopathic or associated with secondary infectious disease, SLE, neoplasia, inflammatory bowel disease, or drug hypersensitivity. The joint disease tends to be cyclic in nature, has a predisposition for smaller distal joints, the carpus and tarsus in particular, and can occur in monarticular (rare), pauciarticular

(uncommon), or polyarticular (common) forms. Radiographic changes, even after many months of joint disease, tend to be minimal or non-existent, and are limited to soft tissue swelling. Biopsies of the synovial membranes show a non-villous hyperplasia, sparse mononuclear cell infiltrates, neutrophil infiltrate, and fibrin exudation. Marginal erosions and pannus formation are not prominent features in these diseases.

Regardless of the overlying or underlying disease processes that lead to the arthritis, the joint disease is believed to be due to deposition of immune complexes in the synovial membrane with resultant type III immune (i.e., vasculitis) reaction. In idiopathic non-deforming arthritis, the origin and nature of the antigen in the complexes are unknown; the antigens in SLE are of nuclear origin; the antigen in enteropathic arthritis probably originates from the inflamed bowel; while antigens in arthritis secondary to chronic infectious disease or neoplasia originate from the offending microorganisms or from the tumor cells.

4.2.5.1. Idiopathic non-deforming arthritis. Idiopathic non-deforming arthritis is by far the most common disorder of dogs manifesting immune-mediated arthritis (Pedersen et al., 1976b; Pederson and Pool, 1978). It is termed idiopathic because there is no evidence of underlying diseases. This disorder occurs most predominantly in purebreds. Although there is a feeling that medium and larger sporting-type breeds are more affected, the condition has also been observed in many small breeds. A possible association between immune-mediated thyroiditis and hypothyroidism may exist; the numerous breeds that suffer from hypothyroidism are among the breeds most commonly affected with idiopathic non-deforming arthritis. An idiopathic non-deforming polyarthritis has also been recognized in cats, but much less frequently than in dogs.

The disease is usually manifested in animals from 1 to 6 years of age, but is not unusual in puppies, adolescents and older dogs. The initial presenting history is one of cyclic fever, during which malaise, anorexia, lameness, or generalized stiffness are noted. The fever is most pronounced in dogs with polyarticular disease and least pronounced in animals with monarticular involvement. In severely affected dogs, periods of remission are usually incomplete, in which case the disease can be very debilitating. Generalized muscle atrophy and disproportionate atrophy of the temporal and masseter muscles are frequently seen. This atrophy is due in part to disuse, but in many cases the disease process also involves the muscles or nerves.

During the most severe stages of the disease, swelling and heat in distal joints are sometimes detected. Generalized lymphadenopathy may be noted in varying degrees. During periods of disease activity, a leukocytosis with neutrophilia and hyperfibrino-genemia is often observed. Polyarticular involvement is the most common presentation, with the dogs showing generalized stiffness and reluctance to move their spine, tail, or limbs. Toy breeds, which often have severe generalized arthritis, can become virtually immobile, making it difficult to tell whether the joints are the source of the problem, or whether the immobility is due solely to depression.

Radiographic abnormalities are usually not present, except for an increase in the amount of periarticular soft tissue due to inflammation or fibrosis. If the disease is present for many months without treatment, however, some mild degenerative changes can occur in the joints. Persistent hyperemia of the synovium can lead to severe periarticular

periosteal bone proliferation in rare individuals. These radiographic abnormalities can lead to a mistaken diagnosis of degenerative joint disease (DJD). Obviously, such misdiagnosis will greatly influence the type of therapy selected. It is important, therefore, to always take a sample of synovial fluid from dogs with DJD changes that are atypical for that disease.

Diagnosis is made by consideration of the clinical history of an antibiotic-unresponsive cyclical fever, malaise, and anorexia, upon which is superimposed stiffness or lameness. The cyclical nature of the disease complicates diagnosis because affected dogs may appear to respond favorably to antibiotic therapy when in fact the improvement is due to coincidental cyclic variation of the inflammatory process.

Synovial fluid analysis is imperative, even if there are no signs of joint pain, swelling and reddening (Pedersen, 1978). Synovial fluid from affected individuals contains from 5000 to 100 000 or more white cells per μl. The predominant cells in the fluid are the neutrophils; these cells appear non-toxic and with normal granulation. The fluid is sterile for bacteria, viruses, *Mycoplasma*, and *Chlamydia*. Serologic abnormalities such as the LE cell phenomenon, antinuclear antibody, and rheumatoid factor are absent. Blood cultures are negative for bacteria, and there are no signs of primary infectious processes in other areas of the body.

Treatment involves the use of glucocorticoids alone or in combination with more potent immunosuppressive drugs (see Section 4.2.5.2). A complete remission of signs can usually be achieved. From 30% to 50% of the dogs will have recurrences of illness after the drug therapy is discontinued.

4.2.5.2. Non-deforming arthritis associated with chronic infectious diseases. A non-deforming arthritis associated with chronic infectious disease has been described in dogs (Pedersen et al., 1976b; Bennett, 1986; van der Wel and Meyer, 1995). This type of arthritis has been associated with subacute bacterial endocarditis; pyometra; discospondylitis; chronic *Actinomyces* infections in the chest, abdomen, or paravertebral musculature; chronic salmonellosis; heartworm disease; urinary tract infections and severe periodontitis. Since these infections are often difficult to pinpoint, the arthritis may be the main or sole presenting complaint. It is important, therefore, to make a thorough search for secondary infections every time a non-erosive type of arthritis is found. This is especially important because immunosuppressive drugs are contraindicated in animals with underlying infections.

Joint involvement in this type of disorder is usually monarticular or pauciarticular, and has a predisposition for the carpal and tarsal joints. Since the organisms involved in the primary disease process cannot be identified in the synovial membrane, it is likely that the joint disease is also of immune complex origin. A similar relationship between a sterile arthritis and chronic infections in other parts of the body was recognized much earlier in man (Coggeshall et al., 1941).

4.2.5.3. Non-deforming arthritis associated with other immune disorders

4.2.5.3.1. Systemic lupus erythematosus. A non-deforming polyarthritis is the most common presenting clinical feature of canine SLE. Like virtually all immune-mediated

arthritides, care must be taken to exclude underlying causes of disease, e.g., infections, drugs, neoplasia. A non-erosive polyarthritis has been observed in subacute bacterial endocarditis (SBE) in man and dogs, and indeed the entire syndrome of SBE can mimic SLE. Two dogs described by Bennett (1986) best document this point. Chronic bacterial endocarditis can lead to continuous low-grade damage to parenchymal organs, high levels of circulating immune complexes, and a heightened responsiveness of the hosts immune system. In man and animals this may result in the production of numerous autoantibodies, including antinuclear antibody and rheumatoid factors. Antinuclear antibodies result from chronic nucleoprotein release and heightened immunologic responsiveness, and rheumatoid factors are made in response to persistent immune-complex production. This phenomenon is important, because if such animals are mistakenly diagnosed as having SLE or rheumatoid arthritis, they will be treated with immunosuppressive drugs, with potentially serious consequences.

4.2.5.3.2. Immune-mediated polyarthritis and meningitis. A pauci- or polyarticular arthritis can be an accompanying feature of immune-mediated meningitis, although signs referable to joint inflammation are almost always masked by the signs of the meningitis. The exception is in Akita puppies (Dougherty et al., 1991); both meningitis and polyarthritis are clinically apparent.

4.2.5.3.3. Familial mediterranean fever. Familial Mediterranean Fever is a human disorder characterized by recurrent fever of unknown origin, renal amyloidosis, and evidence of peritonitis, pleuritis, and/or synovitis. A syndrome thought to be similar to familial Mediterranean fever has been described in Chinese sharpei dogs (Rivas et al., 1992). Whether or not the syndrome is the same as the human disorder remains to be determined. Diseased dogs showed elevated levels of IL-6 in serum and hypergammaglobulinemia when compared to normal controls. Affected dogs that were two years old or older frequently suffered from renal failure associated with amyloidosis and swollen joints.

4.2.5.3.4. Juvenile cellulitis and arthritis. Four of 15 dogs diagnosed with juvenile cellulitis (juvenile pyoderma, puppy strangles) had signs of joint pain in addition to the characteristic facial inflammation and submandibular lymphadenopathy (White et al., 1989). Synovial fluids from three of the four dogs with joint pain revealed suppurative arthritis and were negative for bacterial growth on culture. Concurrent treatment with antibiotics and prednisone was highly effective in curing both facial and joint lesions.

4.2.5.4. Non-deforming arthritis associated with neoplasia. A sterile polyarthritis has been observed in some dogs and cats with overt or latent neoplastic processes in other parts of the body (Pedersen et al., 1976b; Bennett, 1986). As such, the signs of polyarthritis may precede the signs of cancer or may be a minor to major component of the overall disease syndrome.

4.2.5.5. Enteropathic arthritis. Enteropathic arthritis is frequently associated with disease like ulcerative colitis and regional enteritis of man. The cause of the arthritis is unknown,

but it is thought that either the bowel and joint disease share a common etiology, or antigenic products released into the blood from the inflamed bowel have some effect on the synovium. Hepatopathic arthropathy, which has been seen in several dogs with chronic active hepatitis and cirrhosis, is also a type of enteropathic arthritis. In this disease, antigenic material from the bowel probably gains access to the general bloodstream, because it is not being removed from the portal blood by the reticuloendothelial tissue of the liver.

Polyarthritis in dogs with ulcerative colitis and more fulminating enterocolitis has been recognized (Pedersen et al., 1976b). In addition, a small percentage of the dogs with idiopathic polyarthritis have problems with flatulence, occasional vomiting, and eventual gastric torsion; the latter indicates some degree of preexisting motility problem.

4.2.5.6. Plasmacytic-lymphocytic gonitis. A synovitis is observed in 10% or less of dogs operated on for cranial cruciate ligament (CCL) rupture. This subclass of dogs tends to be younger than the usual dog with CCL ruptures and they are often purebred animals of medium-sized breeds such as Rottweilers. It leads to pronounced joint laxity and instability, often manifested by cruciate ligament damage and instability. The synovium is grossly thickened, edematous, reddish-yellowish tinged. The synovial fluid is cloudy, less viscous, yellow tinged, and contains from 5000 to 20 000 white cells per μl, with 10–40% of these being neutrophils. Unlike other inflammatory joint diseases, the predominant cell is often a small lymphocyte. The fluid is sterile for known microorganisms. Radiographic changes, when present, are minimal and include soft tissue swelling and periosteal proliferative changes. Erosive changes are absent or slight. These features may relate to the pre-existing instability due to ligament weakness. Synovial biopsies show an intense lymphocytic-plasmacytic infiltrate and synovial hypertrophy that is sometimes villous. Subchondral erosions are minimal or absent.

The etiology of this disorder is unknown, and it is still uncertain whether the peculiar synovitis leads to the cruciate ligament laxity/rupture or whether it is merely a secondary immune reaction by the host to the degenerating ligament. In favor of the latter etiology, CCL reconstruction is often curative by itself in these dogs. Significantly increased levels of serum anti-native collagen type II antibody, as assessed by ELISA, have also been measured in dogs with diverse joint diseases such as rheumatoid arthritis, infective arthritis, primary DJD and CCL rupture (Bari et al., 1989). In favor of the cruciate disease being secondary to some immune disorder:

1. proper immunosuppressive drug therapy early in the course of the disease often renders affected animals sound;
2. the subset of dogs with this form of CCL rupture are different from the older, sedentary, overweight spayed female dogs that develop classical ligament degeneration;
3. breeds of dogs that suffer from this disorder tend to be the same breeds that are afflicted by other immunologic diseases; and
4. inflammation can sometimes be detected in joints other than the affected stifles.

Regardless of the ultimate cause, it would be prudent to treat atypical cases of CCL rupture with inflammatory synovial fluids medically as early in the disease course as

possible, and to augment or substitute medical treatment with surgery in more advanced cases or in animals that fail to respond medically. Medical treatment would be the same as for other immunologic arthritides.

4.2.5.7. Drug-induced arthritis. Drug-induced vasculitides, usually manifested by acute skin and joint involvement, are becoming increasingly more common in dogs, especially as the proportion of purebred animals increases (Cribb, 1989; Giger et al., 1985; Grondalen, 1987; Harvey, 1987). The most common drug hypersensitivity is to sulfonamides, although the authors have observed identical disease after the use of penicillins, erythromycin, lincomycin, and cephalosporins. Drug reactions involve the deposition of drug-antibody complexes around blood vessels in different areas of the body. The drug may act directly as an antigen or may combine with host proteins as haptens to form neoantigens.

Polyarthritis is only one feature of the disease syndrome; fever, lymphadenopathy, and various types of macular-papular or bullous type hemorrhagic rashes frequently accompany the disease. Joint fluids contain large numbers of non-degenerative neutrophils. The first sign of the allergic reaction tends to occur from 10 to 21 days after drug treatment is initiated for some other condition. This is the time period during which sensitization occurs. Alternatively, drug reactions may occur within hours of administering a drug that had been used safely in the past. Affected dogs become acutely febrile, stiff and sore, and develop a generalized rash. The rash can take several forms, but individual lesions on the skin are clearly of a vascular origin with hemorrhagic blistering, focal necrosis with hemorrhage, severe focal petechiation, etc. Additional features may include lymphadenopathy, polymyositis, anemia, glomerulonephritis, focal retinitis, leukopenia, and thrombocytopenia. The diagnosis is readily apparent, providing the clinician is astute enough to connect drug treatment with the onset of an acute vasculitis syndrome involving primarily skin and joints. This linkage may be missed, especially if it is assumed that the condition at hand is merely an extension of the condition for which the treatment was prescribed. There is a rapid resolution of signs upon discontinuation of drug treatment, although whirlpool baths and glucocorticoids may be required to speed the healing of the skin lesions.

4.2.5.8. Treatment of immune-mediated arthritides. The first imperative is to determine whether or not the arthritis is associated with some significant underlying disease process. When inciting diseases are present, treatment should be concentrated on the underlying cause first and the immune arthritis second. The objective of therapy should be complete remission, which correlates with the disappearance of synovial fluid abnormalities and not just a return to some perception of 'outward normalcy'. Allowing the joint disease to slowly simmer during inadequate treatment can lead to secondary DJD over a period of months or years. Moreover, accepting less than complete remission does the animal an unkindness, because even greater clinical improvement can be expected with total control.

It is important to use synovial fluid analysis to determine when complete remission has been achieved. Dogs may return to near clinical normalcy on treatment, but yet still demonstrate mild synovial inflammation. If low-grade inflammation is allowed to persist,

DJD will gradually occur and complicate the overall clinical picture. It is prudent, therefore, to select several joints that initially showed inflammatory changes and to reexamine fluid from these joints periodically during drug therapy.

A clinically acceptable reduction of joint inflammation can be achieved with glucocorticoids alone in about one-third of cases. A higher percentage can be treated with glucocorticoids alone if the dosage is kept high and the therapy continued over long periods of time. The side-effects of such long-term high-dose therapy are so great, however, that combination drug therapy is preferable.

If, during drug therapy, there is a drastic change in the clinical appearance of the lameness, immediate reevaluation of the status of the joint disease is imperative. The authors have seen dogs that developed severe septic arthritis months after being treated for immune-mediated arthritis when there was an undiagnosed underlying endocarditis or pyelonephritis. It is a grave mistake to reinstitute or intensify drug therapy on the presumption that it is the same disorder.

4.2.6. Immune-mediated meningitis

Immune-mediated meningitis is an increasingly common disorder of dogs. The condition has been also called periarteritis nodosa, and resembles Kawasaki syndrome of humans (Felsburg et al., 1992; Tipold et al., 1995). It is particularly common in adolescent or young adult Beagles, Pointers, Boxers, Akitas, Weimeraners, and Bernese Mountain dogs. It is uncommon in mongrels. The severity and chronicity of disease varies among the breeds.

The clinical signs in Beagles, Pointers, Weimeraners, and Boxers consist of cyclic bouts of fever, severe neck pain and rigidity, reluctance to move, and depression (Albassam et al., 1989; Hayes et al., 1989; Poncelet and Balligand, 1993; Ruben et al., 1989; Snyder et al., 1995; Spencer and Greaves, 1987). Each attack lasts from 5 to 10 days, with intervening periods of complete or partial normalcy lasting a week or more. Cerebral spinal fluid collected during an attack has an elevated protein and neutrophil content; fluid taken between attacks is normal. The causative lesion in Beagles, and in probably in other breeds as well, is an arteritis of the meningeal vessels and occasionally of other tissues as well, e.g., the synovium. The disease is often self-limiting over several months; attacks become milder and of longer intervals between. Glucocorticoid therapy will help minimize the severity of attacks.

A more severe form of immune-mediated meningitis is seen in young Bernese mountain dogs. The disease in the Bernese mountain dogs is somewhat cyclical, but there is incomplete resolution in intervening periods. Cerebrospinal fluid abnormalities resemble those of the disease in these other breeds. The condition is less likely to be self-limiting than in the above breeds and often requires long-term high dose glucocorticoid therapy to maintain the animals in a comfortable state.

A syndrome of meningitis, usually associated with polyarthritis, is seen in Akitas as young as 12 weeks of age (Dougherty et al., 1991). The animals show severe, but somewhat cyclical, bouts of fever, depression, cervical pain and rigidity, and generalized stiffness. Affected animals grow at a slower rate and often appear unthrifty. The condition responds poorly to glucocorticoid and combination immunosuppressive therapy, and most animals are euthanitized as early adults. A milder and more drug responsive form of the

disease is seen in older Akitas. The condition in older Akitas may be associated with pemphigus foliaceous, uveitis, and plasmacytic lymphocytic thyroiditis.

4.2.7. Immune-mediated renal disease

4.2.7.1. Linear deposition of immune complexes. Goodpasture's disease of humans is manifested by immune-mediated glomerulonephritis, nephrotic syndrome, and pulmonary hemorrhage. The glomerular lesions are associated with the binding of anti-glomerular basement membrane antibodies to the glomerular basement membrane. A linear type of antibody deposition is seen by IFA. Pulmonary lesions are associated with a breakdown of alveoli associated with the deposition of antibody in the alveolar basement membranes. A single dog with a condition that histologically and clinically resembled Goodpasture's disease has been recognized.

4.2.7.2. 'Lumpy-bumpy' deposition of immune complexes. Glomerulonephritis associated with granular or 'lumpy-bumpy' immune complex depositions is the most common immune complex disorder of animals. It exists in several histopathologic forms and is either primary (idiopathic) or secondary in nature. The lesions result from irregular deposition of immunoglobulin and complement in the subepithelial or subendothelial side of the glomerular basement membranes. Lumpy-bumpy deposition is consistent with circulating pre-formed immune complexes rather than in situ or anti-basement membrane reacting antibodies.

Glomerulonephritis is either the sole or primary manifestation (idiopathic glomerulonephritis), or is a secondary feature of some other immune-complex generating disorder. Dogs with secondary glomerulonephritis almost always present for signs of their overlying disease and the kidney problems are diagnosed secondarily. Glomerulonephritis has been described secondary to chronic infectious diseases, such as babesiosis (Wozniak et al., 1997), infectious canine hepatitis (Hervaas et al., 1997), dirofilariasis (Nakagaki et al., 1993), ehrlichiosis (Codner et al., 1992), leishmaniasis (Nieto et al., 1992; Poli et al., 1991), and bacterial endocarditis (Macdougall et al., 1986), and to non-infectious conditions such as chronic congenital portosystemic shunts (Tisdall et al., 1996) and congenital complement deficiency (Ameratunga et al., 1998; Blum et al., 1985).

Dogs with idiopathic glomerulonephritis present mainly for signs of their kidney disease. As in humans, juvenile and adult-onset forms of the disease occur in dogs. A juvenile nephropathy has been reported in a Dalmatian (Dixon, 1997). A familial glomerulopathy, associated with membranoproliferative glomerulitis and interstitial nephritis, was observed in Bernese mountain dogs (Minkus et al., 1994; Reusch et al., 1994a, b). Dambach et al. (1997) described 49 cases of a rapidly progressive and highly fatal glomerulonephritis in dogs; affected animals were younger (5.6 ± 2.6 years) than other dogs with idiopathic glomerulonephritis (7.1 ± 3.6 years) and Labrador and Golden retrievers were five times or more over represented. The glomerular disease was mainly membranoproliferative and was associated with subendothelial IgG, IgM and C3 deposition. Although this disease has been linked to Lyme borreliosis exposure and vaccination, silver stains failed to show a strong relationship between spirochetes in the kidneys and lesions development.

Many dogs with glomerulonephritis present with nephrotic syndrome—the triad of proteinuria, hypoproteinemia, and peripheral edema. If the glomerular disease is significant, there is also a concomitant loss of nephrons and signs of renal failure (elevated serum creatinine, azotemia, isosthenuria and polyuria/polydypsia). Dogs with glomerulonephritis and nephrotic syndrome are often hypercoagulable (due to renal loss of anticoagulants) with elevated serum fibrinogens; sudden death due to thrombosis of pulmonary arteries is a serious problem in such animals. Dogs with secondary glomerulonephritis almost always present with signs of their overlying illnesses and the glomerular disease is detected as an incidental finding during the clinical work-up or at post-mortem examination.

The diagnosis of glomerulonephritis is made on the basis of clinical signs (weight loss, peripheral edema polyuria/polydypsia) and laboratory findings (hypoproteinemia and hyperproteinuria), and confirmed on renal biopsy. Glomerular changes can be classified in most cases by conventional light microscopy and the pattern of immune complex deposition determined by immunofluorescene or immunoperoxidase staining. All of the WHO human categories of glomerular disease have been observed in dogs, i.e., focal, diffuse mesangial proliferative, diffuse endocapillary proliferative, mesangiocapillary, diffuse crescentic, diffuse sclerosing, amyloid, and unclassifiable (Macdougall et al., 1986). Immune complexes consist of IgG, IgM (Minkus et al., 1994), IgA (Harris et al., 1993; Miyauchi et al., 1992), or combinations of the three, and complement. IgG and C3 are more apt to be associated with dense deposits, while IgA and IgM are observed in animals with dense or minimal deposits (Koeman et al., 1987). Membranous and mesangial proliferative changes vary in severity, and in exceptional cases, lesions are unapparent upon light microscopic examination and may even be minimal when viewed by electron microscopy (Vilafranca et al., 1993). These latter dogs have a condition analogous to 'minimal change disease' of humans.

If there is an overlying or underlying disease condition, the emphasis should be placed on treating that disease. There is some debate on whether dogs with GN should be treated conservatively with low protein diet and blood pressure control (Lulich et al., 1996), or vigorously with the addition of immunosuppressive drugs. Conservative therapy is often favored because of the relatively high incidence (about 20%) of spontaneous remission (Biewenga and Gruys, 1986), and to the poor response and side-effects of prolonged immunosuppressive drug therapy. Cyclosporine treatment was found to offer no improvement over non-treated animals in treatment of glomerulonephritis and was associated with a number of side-effects (Vaden et al., 1995). There is no question that some dogs, especially younger animals with minimal glomerular changes and acute onset nephrotic syndrome, will self-cure after several months of conservative treatment (diet change with or without glucocorticoids). However, the disease in other dogs will not self-cure and vigorous treatment will slow the course of renal disease. Glucocorticoids alone are usually not effective, and combination therapy with glucocorticoids and cytotoxic drugs such as cyclophosphamide or azathioprine is required. Unfortunately, most dogs with glomerulonephritis present late in their disease course when treatment is minimally effective. The mean survival of one large group of dogs with glomerular disease (glomerulonephritis and renal amyloidosis) was 28 days (Cook and Cowgill, 1996).

4.2.7.3. Renal amyloidosis. Amyloidosis is technically not an immune complex disease, and does not fit into the original Gel and Coomb's classification scheme. Amyloidosis occurs under the same conditions as immune-mediated glomerulonephritis. It is idiopathic or secondary to chronic antigen stimulating diseases, chronic inflammatory diseases, chronic infectious diseases (MacIntire et al., 1997), other immune diseases such as rheumatoid arthritis or juvenile polyarteritis (Snyder et al., 1995), dermatomyositis (Hargis et al., 1989), congenital complement deficiency (Blum et al., 1985), neoplasia (Geisel et al., 1990; Platz et al., 1997), and senility (Johnson et al., 1992; Shimada et al., 1991, 1992; Uchida et al., 1990, 1991). Genetics may play an important role in idiopathic amyloidosis; it has been described in Chinese shar pei dogs (DiBartola et al., 1990; Dubuis et al., 1998; Loeven, 1994; Rivas et al., 1993), Beagles (Bowles and Mosier, 1992), and in English Foxhounds (Mason and Day, 1996).

Amyloid is a complex β-pleated protein and several different serum components can contribute to its make-up (Sellar et al., 1991). In most cases, amyloid consists mainly of reaggregations of the variable regions of immunoglobulin light chains. Conditions associated with an increased production and catabolism of immunoglobulin, therefore, are most likely to be associated with this form of amyloid (amyloid-light chain or AL). How the intact immunoglobulin light chains are converted into amyloid fibrils is not known, but it may involve catabolism of immunoglobulin or immunoglobulin subunits by macrophages. Amyloid can also be made up of non-immunoglobulin proteins, so-called amyloid of unknown origin or amyloid A (AA). A small proportion of AL or AA consists of a plasma component related to $\alpha 1$ globulin.

Amyloidosis in man and animals has been classified as either primary (idiopathic) or secondary to chronic infectious diseases, immunologic diseases, neoplasia, or senility. There is a tendency, however, to classify amyloidosis in man according to the chemical composition of the amyloid.

The following classification has been used in man, and appears to be similar in animals:

Type	Clinical form	Site of deposition	Chemical type of Fibril
Familial		Liver, spleen, kidneys, adrenals	AA
Generalized	Idiopathic, plasma cell dyscrasia, secondary to chronic infections and inflammations	Many organs, kidney in particular	AL, AA
Localized	Lichen amyloidosis	Skin	AD (amyloid-dermal)
	Endocrine-related	Endocrines (thyroid, pancreas)	ARE (amyloid-endocrine)
Senile		Heart, brain	AS_c, AS_b

Amyloidosis in dogs usually involves the kidneys; beta amyloidosis of the brain in aged dogs has been described less commonly (Weegiel et al., 1996). Renal amyloidosis is associated with heavy proteinuria, nephrotic syndrome, and eventually renal failure. Like

glomerulonephritis, primary forms of renal amyloidosis present mainly with nephrotic syndrome while secondary forms are often associated with underlying or overlying disease syndromes. Of the various glomerulopathies in dogs, amyloidosis and membranous glomerulonephritis are the most likely to be associated with nephrotic syndrome, azotemia, hypercoagulation and thrombotic disorders (Biewenga and Gruys, 1986; Cook and Cowgill, 1996).

If secondary, and the primary cause can be corrected, some reversal of signs may occur. Several dogs with idiopathic renal amyloidosis have been treated with DMSO orally; the substance is taken into the blood and secreted in the kidneys. The objective is to dissolve amyloid deposits and to prevent further amyloid deposition (Spyridakis et al., 1986). However, it is hard to get animals to ingest sufficient quantities and there is a heavy garlic odor. DMSO treatment is not always effective and appears to prolong survival rather than curing the disease. Overall, renal amyloidisis in dogs carries a poor prognosis with a mean survival time of less than one month (Cook and Cowgill, 1996).

4.2.8. Dermatomyositis

Dermatomyositis has been described in Collie, Sheltie and Collie/Labrador retriever crosses (Hargis et al., 1986). Cutaneous, muscular and vascular lesions were noted in juvenile to adult animals. Gross lesions were most severe on the head and distal extremities. Microscopic lesions included myositis in the esophageal musculature; arteritis in skin, muscle, bladder and spermatic cord; lymphoid hyperplasia, especially of peripheral nodes; and a mild lymphocytic thyroiditis.

5. Type IV reactions (cell-mediated immunity)

5.1. Mechanism of type IV immune reactions

Cell-mediated immunity is mediated by two cell types, lymphocytes and macrophages. It is an evolutionary step between phagocytosis and humoral immunity. Natural killer (NK) cells appeared first, followed thereafter by specific thymus-derived lymphocytes (T-cells). Cell-mediated immunity functions mainly against body cells that have been rendered foreign, either by malignant transformation or from intracellular microorganisms, or against invasion by cells from other individuals of the same (allogeneic cells) or different (xenogenic) species.

NK cells are a subpopulation of lymphocytes that bear neither T- or B-cell markers, hence the alternative name of 'null cells'. NK cell activity rests within the large granular population of blood lymphocytes. NK cells have primitive receptors on their surface that can non-specifically identify many types of antigenically altered host cells (malignant cells, infected cells). Because a single NK cell can recognize a wide spectrum of abnormal cells, NK cell immunity is not antigen specific. Therefore, NK cell activity is considered to be an integral part of innate, rather than adaptive, immunity. Most in vitro assay procedures for NK cell activity involve killing of tumor cell targets. NK cells recognize foreign or altered cells during their wanderings through the body; the same cell being capable of recognizing a wide range of abnormal cells. NK cells touch membranes

of abnormal cells and kill them by releasing specific lymphokines. NK cell-mediated killing can be enhanced by antibodies specific for abnormal surface antigens on malignant or antigenically altered cells, a process known as antibody dependent cellular cytotoxicity (ADCC). This is an example of how a more primitive form of immunity can be upgraded by the subsequent evolution of antigen specific humoral immunity. T-cells, which evolved for cell-mediated immunity, were also used later for control and regulation of B-cell immunity.

T-cell-mediated immunity is mediated by two subclasses of cells, those that bear the CD4 cell surface antigen and those that possess the CD8 cell surface antigen. The CD4+ T-cells 'help' B-cells produce antibody and are often referred to as helper cells. They are also important for the generation and maintenance of immunologic memory. CD8+ T-cells function to suppress B-cell activity and are called suppressor cells. These cells are also important as effectors of adaptive cell-mediated immunity. T-cells have evolved immunoglobulin-like receptors on their surfaces. Unlike NK cells, each T-cell possess receptors to a single antigenic epitope. Therefore, hundreds of thousands of antigen specific T-cell subpopulations exist in the body. B-cell (humoral) immunity first appeared in higher fish and is a natural evolutionary step from T-cell immunity. In T-cell immunity, immunoglobulin-like receptors are firmly anchored to the cell surface, while in B-cell immunity, the 'receptors' are also excreted free into the surrounding body fluids as antibodies.

T-cell-mediated cytotoxicity is triggered when cell surface antigens on either a foreign (allogeneic, xenogeneic) or altered host cell is recognized by its corresponding antigen specific T-cell. Initial recognition leads to the elaboration of numerous lymphokines/cytokines. Some of these lymphokines may have non-specific anti-microbial activity, such as interferons. Other cytokines induce a clonal expansion of a subpopulation of T-cells that bear the identical antigen receptor. Some of these proliferating T-cells become antigen-specific effector or killer cells. T-effector cells can recognize and kill the abnormal cells by bringing themselves into intimate contact and releasing cytotoxic lymphokines. Because macrophages are a favorite reservoir for microbial growth or ingest organisms as part of their phagocytic function, they are an important target for T-cell responses. T-effector cells can either kill infected macrophages directly, activate surrounding macrophages to assist in direct cell killing, or 'instruct' the macrophage to activate itself and to destroy any microbes that it might contain. The latter two processes are most important for intracellular infection with viruses, while the latter is more important for macrophages containing complex microbes such as bacteria, protozoa, parasites or fungi.

Host cells are rendered foreign when specific foreign antigens are elaborated on their surfaces, and are thus rendered targets for attack by specific T-cells and activated macrophages. Malignant cells may express new antigens on their surface; these antigens may be unique to the body or were expressed only during embryogenesis and prior to the time that self-tolerance is induced. Cells that are infected with microbes are also rendered foreign. Foreign proteins produced within the cells by the microbe are transported to the cell surface and expressed on the major histocompatibility complex-I (MHC-I). Host cells may also be rendered foreign when their surface antigens are chemically altered, as in the case of contact hypersensitivities to plastics, drugs, or other substances. In rare cases,

normal cells can come under attack by killer T-cells by mechanisms that are identical to those described for autoantibody responses (Wucherpfennig et al., 1995). These various mechanisms are amply illustrated by the number and variety of immune disorders of dogs that have a type IV immune etiology.

5.2. Diseases involving type IV reactions

5.2.1. Delayed hypersensitivity reactions of skin

Flea allergic dermatitis is interesting in that immediate, intermediate and delayed hypersensitivity reactions can occur in the same animal. In fact, an evolution from one type of immunity to the other was demonstrated in animals almost four decades ago (Benjamini et al., 1961). Immediate reactions are characterized by reactions that appear within 30 min of antigenic challenge, whereas delayed hypersensitivity reactions take 48–72 h to reach peak intensity. The former reaction is characterized by edema, neutrophil, and eosinophil infiltrate, the latter by inflammation and mononuclear cell (Lymphocyte, macrophage, plasma cell) infiltrate.

Poison oak and poison ivy are classic examples of delayed hypersensitivity reactions in humans. The oils of these plants chemically combine with epidermal proteins and result in the formation of neoantigens. Sensitization of people to these altered proteins occurs after the initial exposure. Subsequent exposures lead to a rapid influx of lymphocytes and macrophages into the sensitized skin. The resulting delayed hypersensitivity reaction is essentially an attempt to reject ones own skin. Fortunately, dogs are not sensitive to most plant sensitizers. Analogs of poison oak and ivy are observed in dogs in the case muzzle hypersensitivities associated with eating out of plastic food dishes. Resins present in some plastics will leach into the food and bind with the skin on the muzzle.

5.2.2. Infection granulomas

Granulomatous reactions occurring in response to a number of intracellular infectious agents (tuberculosis, coccidioidomycosis, Histoplasmosis, etc.) are essentially delayed-type hypersensitivity reactions. They result, however, from only partial cellular immunity. Complete cellular immunity results in effective activation of macrophages and destruction or inactivation of intracellular organisms. A complete failure of cellular immunity leads to widespread dissemination of organisms with little attempt on the host to contain the infection. In cases of partial cellular immunity, containment of the organism is incomplete and is manifested by granuloma formation.

5.2.3. Juvenile pyoderma

Juvenile pyoderma is a pronounced submandibular and cervical lymphadenopathy with edema and pustule formation of the muzzle (White et al., 1989). It occurs exclusively in puppies, especially of Brittany Spaniels, Dachshunds and Pointer breeds. Juvenile pyoderma is usually associated with a facial staphylococcal pyoderma. However, the condition seems to result from an inappropriate cellular immune response to the staphylococcal infection. It is treated with both antibiotics and prednisolone. As the dogs get older, their immune responses become more normal and such flamboyant responses are not seen.

5.2.4. Type IV diseases of the joints

5.2.4.1. Canine rheumatoid arthritis. Rheumatoid arthritis is one of the most common and serious immune diseases of humans. It occurs in Juvenile and adult-onset forms. The precise etiology is unknown, but the disease process is of a type IV nature and is directed against antigens within the joint. Whether these antigens are of host origin, or to some yet unknown agent, is unknown. The dense mixed-inflammatory reaction is centered in the synovial membrane and the subchondral bone. Cartilage is undermined by subchondral inflammation and overlain by a pannus of granulation tissue emanating from points of synovial attachment. The net effect is a progressive destruction of cartilage starting at the joint margins; this ultimately leads to joint instability, subluxation, and luxation. The synovial membrane undergoes a characteristic villus hyperplasia with dense infiltrates of plasma cells and lymphocytes. Lymphocytes in the infiltrate, in the human disease, result from an almost clonal expansion of selected CD4+ T-cells which are autoreactive and lack functionally important CD28 surface markers (Martens et al., 1997). However, the role of these cells in the pathogenesis or etiology of human RA is unknown (Breedveld, 1998). The inflammatory changes observed in RA appear to be cytokine produced by macrophage/monocytes and T-cells (Smolen et al., 1996).

Rheumatoid factors are hallmarks of human RA. Rheumatoid factors are auto-antibodies, usually IgM, that are reactive against immune complexed Ig. Rheumatoid factors are present at high titer in about 70% of humans with RA, but are also detected in patients with a wide range of chronic infections. Rheumatoid factor is not pathogenic in itself, because passive transfer of high titered serum does not induce arthritis. It is possible, however, that immune complexes containing rheumatoid factor contribute to the disease by a type III mechanism.

Canine rheumatoid arthritis is a well-documented clinical entity (Halliwell et al., 1972; Liu et al., 1969; Newton et al., 1976; Pedersen et al., 1976a), although uncommon compared to the non-erosive arthritides. The incidence has been approximately two per 25 000 dogs examined at the authors' hospital, which is significantly less than the frequency of RA in people. This disorder occurs mainly in small or toy breeds of dogs as young as eight months and as old as eight years of age. Although rheumatoid arthritis has been categorized as an erosive polyarthritis in this discussion, clinicians in some parts of the world often lump non-erosive polyarthritis with rheumatoid arthritis. In the authors' opinion, this is unjustified because of clear histopathologic, radiographic, and clinical differences between the two types of diseases.

Canine rheumatoid arthritis is manifested initially as a shifting lameness with soft tissue swelling around involved joints. Within several weeks or months, the disease localizes in particular joints and characteristic radiographic signs develop. Joint involvement is more severe in the carpal and tarsal joints, although in individual dogs, the elbow, stifle, shoulder, and hip joints may show similar radiographic signs. Involvement of the apophyseal joints and costovertebral articulations rarely progresses to the point of causing radiographic changes. In exceptional cases, however, involvement of the vertebral articulations can only occur. The disease is often accompanied by fever, malaise, anorexia, and lymphadenopathy in the earlier stages. Renal amyloidosis can be a

complication of long-standing disease that is inadequately controlled (Colbatzky et al., 1991).

The earliest radiographic changes consist of soft tissue swelling and loss of trabecular bone density in the area of the joint. Lucent cyst-like areas are frequently seen in the subchondral bone. The prominent lesion is a progressive destruction of subchondral bone in the more central areas as well as marginally at the attachment of the synovium (Pedersen et al., 1976b). Both narrowing and widening of the joint spaces are identified radiographically as a result of cartilage erosion and destruction of subchondral bone. Subluxation, luxation, and deformation occur most frequently in the carpal, tarsal, and phalangeal joints and occasionally in the elbow and stifle joints. Fibrous ankylosis can occur in advanced cases, particularly in the intercarpal and intertarsal joint space.

Hemograms are either normal or reflect the generalized inflammatory process with a leukocytosis, neutrophilia, and hyperfibrinogenemia (Pedersen et al., 1976b). Serum electrophoresis will often show hypoalbuminemia and variable elevation in α2- and γ-globulins. Rheumatoid factors, i.e., autoantibodies against sterically altered (e.g., immune complexed) antibodies, are the hallmark of human rheumatoid arthritis; they are present at high titer in about three-fourths of affected people. Rheumatoid factors probably serve a function in cross-linking immune complexes and aiding in their clearance by phagocytes. They are detectable, therefore, in many diseases where chronic immune complexing is occurring. Although of definite value in the diagnosis of human rheumatoid arthritis, the value of rheumatoid factor tests in canine rheumatoid arthritis is less certain. Some veterinary researchers have been able to detect rheumatoid factors in a majority of affected dogs, but in only a small proportion of normal dogs and animals with other conditions (Halliwell et al., 1989; Bennett and Kirkham, 1987). In one study, however, rheumatoid factors were detected at comparatively low titer and in only about one quarter of the cases of canine rheumatoid arthritis (Pedersen et al., 1976b). Recent studies have also confirmed that rheumatoid factors are present at only low titer and in only about one-fourth of dogs with rheumatoid arthritis; they are also present at similar titer and incidence in unclassified polyarthritis, heartworm disease, pyometra, leishmaniasis and systemic lupus erythematosus (SLE) (Chabanne et al., 1993; Nielsen, 1992). Antinuclear antibodies are sometimes detected in dogs with rheumatoid arthritis, but not as frequently as in SLE.

Synovial fluid changes are indicative of an inflammatory synovitis, with an elevated total cell count, a high proportion of neutrophils in the synovial fluid cell population and a variable decrease in the quality of the mucin clot. Ragocytes (neutrophils that have ingested immune complexes), as described in human rheumatoid arthritis, are not usually seen. A characteristic finding in canine rheumatoid arthritis is the presence in synovial fluid of mononuclear cells containing IgG, with only occasional cells containing C3 protein (Pedersen et al., 1976b). These mononuclear cells may be producing the immunoglobulin or ingesting it from the synovial fluid.

The characteristic pathologic lesions consist of a villous hyperplasia of the synovial membrane and lymphoid and plasma cell infiltrates in the synovium. Infiltration cells destroy articular cartilage starting at the margins of the joint. A pannus of granulation tissue also invades the surface of the cartilage. In the central regions of the joint, cartilage

destruction is caused by a pannus arising from granulation tissue in the underlying marrow cavity. Ankylosis in advanced lesions is not uncommon in the intercarpal and intertarsal joints. The dense lymphoid and plasma cell infiltrate in the synovium and the destruction of articular cartilage differentiate rheumatoid arthritis from the synovitis seen in the non-erosive types of arthritis. The presence of large numbers of lymphocytes and plasma cells in the synovium suggest the presence of a local antigenic stimulus and a type IV or delayed-type hypersensitivity reaction; the nature of the antigen that is evoking this reaction is unknown.

Canine rheumatoid arthritis responds only temporarily to systemic corticosteroids. Aspirin has no appreciable therapeutic benefits in the author's hands, probably because the disease is much more severe and rapidly progressive in dogs than in man. If the condition is recognized before severe joint damage occurs, it can usually be arrested with combination immunosuppressive drug therapy. This type of therapy will be covered in detail in the discussion of drug therapy of immune-mediated arthritides. In dogs with advanced deformities, immunosuppressive drug therapy may have to be combined with arthrodesis of selected joints. Arthrodesis is not warranted if the disease process cannot first be successfully halted with drug therapy.

5.2.4.2. Polyarthritis of greyhounds. A semi-erosive polyarthritis in greyhounds occurs in different parts of the world (Castelli, 1969; Huxtable and Davis, 1976; Woodard et al., 1991). Disease appears in animals from 3 to 30 months of age and most frequently attacks the proximal interphalangeal, carpal, tarsal, elbow, and stifle joints. The shoulder, hip and atlanto-occipital joints are less frequently involved. A tenosynovitis may be an accompanying feature. The synovial membrane is edematous and hyperemic in the early course of the disease, and may be covered with a fine layer of fibrin. The synovial fluid is cloudy and yellowish, and often contains fibrin tags. In later stages, a lymphocyte and plasma cell infiltrate is seen in the synovial lining. Peripheral lymph nodes are enlarged and hyperactive. Pannus formation and marginal subchondral erosions are seen to a limited extent. Destruction of articular cartilage occurs in some joints but often is not associated with pannus formation. Gross deformities and radiographic changes are not as apparent as those seen in canine rheumatoid arthritis but appear more pronounced than those described for non-erosive joint disease.

Mycoplasmal and bacterial isolations have usually been unsuccessful and dogs are serologically negative for *Erysipelothrix* and *Chlamydia*. However, *Mycoplasma spumans* has been isolated from a young greyhound with polyarthritis (294). The significance of this single isolate from a polyarthritis of greyhounds remains to be determined.

5.2.5. Glandular diseases involving type IV immunity

A number of glandular disorders of man and animals appear to be involved with immune phenomena, and it is not always clear whether immunologic abnormalities are a cause or effect. Lymphocytic thyroiditis, adrenalitis, and parathyroiditis, and type I diabetes mellitus are endocrine disorders that are related in some way to autoimmunity in dogs (Greco and Harpold, 1994). Immune phenomena are also involved in keratitis sicca, perianal fistulae, and sebaceous gland adenitis.

5.2.5.1. Lymphocytic/plasmacytic thyroiditis. Hypothyroidism is undoubtedly the most common endocrine disorder of dogs. The disorder is caused, in equal proportion, by lymphocytic/plasmacytic thyroiditis or idiopathic atrophy (Kemppainen and Clark, 1994). The former is analogous to Hashimoto's thyroiditis of man and appears to be of autoimmune origin. Thyroid atrophy may not be a separate entity, but rather an end stage of the thyroiditis; 8/8 hypothyroid dogs in one study had lymphocytic thyroiditis on biopsy (Nachreiner et al., 1998). Thyroiditis was also shown to progress to severe atrophy in a study of hypothyroid Beagles (Benjamin et al., 1996). As in humans, hypothyroidism underlies a significant proportion of other immune disorders in dogs, and is seen overwhelmingly in purebreds. The breeds that suffer most from hypothyroidism include Golden retrievers, Beagles, Doberman pinshcers, Akita, Cocker spaniel, and Rottweilers, which are among the breeds that suffer from a range of immune disorders.

In addition to the common finding of lymphocytic infiltrate, fibrosis and atrophy, many dogs with hypothyroidism will have detectable anti-thyroid antibodies. In human Hashimoto's disease, these antibodies are usually against thyroid peroxidase; however, this does not appear to be the case in dogs (Thacker et al., 1995). Rather, dogs with lymphocytic thyroiditis develop antibodies to T3, T4 and thyroglobulin to varying degrees (Nachreiner et al., 1998). Antibodies to thyroglobulin occur predominantly in IgG1, IgG3 and IgG4 subclasses (Day, 1996a, b, c).

Clinical signs of hypothyroidism in affected dogs are, in order of frequency, obesity, seborrhea, alopecia, weakness, lethargy, bradycardia, and pyoderma (Panciera, 1994). Neurologic disorders (Jaggy et al., 1994a, b; Jaggy and Oliver, 1994) and reproductive failure (Johnson, 1994) have also been associated with hypothyroidism in dogs. The diagnosis of thyroiditis and hypothyroidism should take into account clinical signs, breed predisposition, and evidence of decreased levels of triiodothyronine (T3) and thyroxine (T4), increased levels of TSH, and the presence of antibodies to T3, T4 and thyoglobulin. Some clinical researchers feel that post-TSH stimulation levels of T4 are the single most diagnostic test (Jaggy and Oliver, 1994). The presence of autoantibodies to T3 and T4 cause serum T3 and T4 concentrations to appear falsely high; however, free T4 is non-detectable when measured with a dialysis assay (Kemppainen et al., 1996). Comparative studies of several tests demonstrated that both serum free T4 and TSH concentrations should be used in reaching a diagnosis; although free T4 tests were highly sensitive, greater specificity was obtained by combining the two assays (Peterson et al., 1997). The TSH test, although very specific, lacks sensitivity; about one-fourth of hypothyroid dogs will have values in the normal reference range. Antibody assays for thyroglobulin have also been found to be very sensitive and specific in diagnosing dogs with lymphocytic thyroiditis, when compared to antibody tests for T3 and/or T4 (Nachreiner et al., 1998).

Dogs with hypothyroidism are usually treated with L-thyroxine, which usually brings about resolution of all clinical signs of the disease.

5.2.5.2. Parathyroiditis and hypoparathyroidism. Parathyroid gland biopsies taken from dogs with acquired hypoparathyroidism often demonstrate changes similar to those observed in dogs thyroid and adrenal glands of dogs with hypothyroidism and Addison's disease.

5.2.5.3. Adrenalitis and hypoadrenocorticoism. A lymphocytic adrenalitis has been associated with adrenal atrophy in dogs and hypoadrenocorticoism (Sadek and Schaer, 1996). The disease occurs more often in purebreds with a distinct predisposition to Standard poodles and Leonbergers (Smallwood and Barsanti, 1995). The treatment of choice is hormone replacement therapy, especially desoxycorticosterone pivalate (Kintzer and Peterson, 1994; VanZyl and Hyman, 1994).

5.2.5.4. Polyglandular syndrome. Multiple endocrine disorders of immune nature have been described in dogs. Addison's disease associated with megaesophagus, similar to Allgrove's syndrome of people, has been described in a German shepherd dog (Fritzen et al., 1996). A Boxer dog with both hypothyroidism and Addison's disease has also been reported (Kooistra et al., 1995). Two of four Leonbergers with hypoadrenocorticoism were also found to be hypothyroid (Smallwood and Barsanti, 1995). The combination of adrenal- and thyroid-insufficiency is known as type II polyglandular autoimmunity or Schmidt's syndrome in humans.

5.2.5.5. Insulin dependent Type I diabetes mellitus. Insulin dependent Type I diabetes mellitus of humans is a polygenic autoimmune disease characterized by infiltration of the islets of Langerhans with activated lymphocytes and the production of islet cell antibodies (Elie and Hoenig, 1995). Type I diabetes mellitus is also a common disease in dogs and may be a spontaneously occurring model of the human disorder (Greco and Harpold, 1994; Saei and Gouin, 1997).

5.2.5.6. Keratitis sicca. Keratitis sicca refers to corneal infections that occur secondary to inadequate tear formation. The ultimate cause is disease of the lacrimal glands, either because of canine distemper virus infection, radiation damage, drugs reactions (Majeed et al., 1987), or immune-reactions. An immune-mediated lacrimal gland inflammation of a type IV nature occurs frequently in dogs, most often in purebreds and with some selectivity towards Cocker spaniels. The latter condition in dogs is sometimes referred to as Sjögren's syndrome, a human disorder associated with immune destruction of salivary glands and a rheumatoid-like arthritis. The application of this term to the canine condition is premature.

   The prognosis for keratitis sicca depends on the underlying cause. Animals on chronic drug therapy, especially sulfas, for other conditions should have their medications discontinued; however, permanent damage may have already occurred. Frequent applications of artificial tears and topical antibiotics is usually required as long as tear production is inadequate. Opportunistic fungal infections must be considered in cases with infections that do not respond to antibiotics (Smedes et al., 1992). In the case of immune-mediated disease, which is the most common cause of keratitis sicca in dogs, the treatment of choice is cyclosporine eye drops, which brings about rapid remission in a majority of cases.

5.2.5.7. Sebaceous adenitis. Sebaceous adenitis is a disease recognized predominantly in Akitas and Standard Poodles (White et al., 1995). The disease is manifested by scaling, alopecia and secondary pyoderma. The target of the disorder are the sebaceous glands and

the inflammation is mainly of the lymphocytic/plasmacytic type. Earlier reports of the disease describe the use isotretinoin or etretinate; about one-half of the dogs had a 50% or greater improvement (White et al., 1995). The treatment of choice, however, appears to be leflunomide in combination with glucocorticoids.

5.2.5.8. Perianal adenitis and perianal fistulae. Inflammation of the circumanal apocrine sweat glands, leading to severe perianal fistulae, occurs predominantly in German Shepherd Dogs, and to a lesser degree, Irish Setters (Killingsworth et al., 1988). The incidence is higher in males than females and is greater in intact as compared to neutered animals. The perianal abnormalities have been associated with histological evidence of colitis as well (Harkin et al., 1996). Perianal lesions vary greatly, even in the same dog, in degrees of fibrosis, severity of inflammatory response and depth of sinus tracts (Killingsworth et al., 1988). Lesions tend to be in the zona cutanea, and hidradenitis with formation of epithelial-lined sinus tracts is apparent in over one-half of the biopsies examined. In another study dealing with anatomic predisposition, the only predisposing cause between German shepherds and other non-affected breeds was in the greater number of apocrine sweat glands in the zona cuneata (Budsberg et al., 1985). Secondary bacterial infection, with a number of different bacteria, is inevitably present (Killingsworth et al., 1988).

   Before the immunologic nature of the disease was discovered, the treatment of choice was often surgical excision of the fistulous tissues, or Glucocorticoids and special diets (Ellison, 1995; Harkin et al., 1996; Holt, 1985; Viehoff and van Slujis, 1993). The accepted treatment is now cyclosporine (Matthews and Sukhiani, 1997; Matthews et al., 1997).

5.2.6. VKH syndrome
   Vogt–Koyanagi–Harada syndrome is a specific genetic disorder manifested by bilateral uveitis, meningitis and depigmenting skin lesions, which was first described in humans of Japanese extraction. The underlying immunologic basis appears to be a cell-mediated immune attack against melanotic tissues (Nakamura et al., 1996; Norose and Yano, 1996; Okada et al., 1996; Rao, 1997), possibly through reaction to the MART-1/Melan-A (Sugita et al., 1996). A VKH-like syndrome has been described in Akita dogs (Reusch et al., 1994a, b; Teifke et al., 1998) and in Huskies, Malamutes, Samoyeds and Chows. Although the lesions of the canine disease bear a superficial resemblance to VKH of people, it remains to be proven that the syndrome in dogs is the same. The eyes are much less likely to be involved in dogs than the skin, while the opposite is true in man. CNS signs are also not seen in dogs with VKH-like disease, but this may be due to the absence of melanocytes in the meninges of dogs compared to people. To further confuse the issue, many of these same breeds may have skin disease that superficially resembles VKH, but on closer examination and testing, the lesions are actually caused by pemphigus foliaceus. Human VKH can be strongly linked to certain HLA-types (-DR1 and -DR4); the exact predisposing HLA types differing somewhat according to the race under study (Arellanes-Garcia et al., 1998; Goldberg et al., 1998; Islam et al., 1994; Shindo et al., 1994; Weisz et al., 1995). The strong breed predisposition in dogs parallels the strong ethnic and HLA type predilection in people.

5.2.7. Type IV immune diseases of the nervous system

5.2.7.1. Granulomatous meningoencephalitis (GME). Granulomatous meningoen-cephalitis (GME) is a progressive neurologic disorder of dogs (Braund, 1985; Glastonbury and Frauenfelder, 1981; Murtaugh et al., 1985; Sarfaty et al., 1986), characterized by a heterogenous inflammatory infiltrate of MHC class II positive macrophages and CD3 positive lymphocytes (Kipar et al., 1998). Dogs with GME tend to have neurologic signs referable to the head (lethargy, nuchal rigidity, anorexia), which progress to caudal fossal abnormalities such as dementia, tetraparesis and profound alterations in consciousness (Sarfaty et al., 1986).

The diagnosis is based on the clinical signs and markedly elevated CSF protein levels and white cell counts; CSF cells consist predominantly of lymphocytes with an admixture of neutrophils (Bailey and Higgins, 1986). Dogs with this disorder respond well to combination therapy with glucocorticoids and leflunomide (Gregory et al., 1998). The nature of the lesions and response to immunosuppressive drug therapy suggest that the disorder is due to a type IV autoimmune reaction.

5.2.7.2. Old dog encephalitis. Old dog encephalitis, or delayed-onset canine distemper virus (CDV) encephalitis, results from cellular immunity directed against virus that persists in the brain long after the initial immune stage of infection. The CDV can be of wild- or vaccine-type (Evans et al., 1991), but is probably mutated in some manner (Shapsak et al., 1987; Tobler and Imagawa, 1984). The condition is analogous to subacute sclerosing panencephalitis (SPE) of humans, a disorder associated with the persistence of a genetically defective measles virus in the brain. It is not surprising that CDV and human measles virus are closely related members of the genus *Morbillavirus*. The lesions of old dog encephalitis are more glial proliferative, diffuse, and spare the cerebellum, while lesions of classic CDV encephalomyelitis tend to be focal, necrotizing, and more concentrated in the cerebellum, brain stem and spine (Vandevelde et al., 1980).

Old dog encephalitis is a slowly progressive encephalitis that occurs in adult, vaccinated dogs, and is a differential in other types of chronic encephalitides such as GME. Signs are usually significant, although a mild encephalitis with the predominant sign being a loss of smell has been reported in a Beagle (Simpson and Myers, 1987). Dogs with delayed-onset CDV encephalitis, unlike animals with acute demyelinating CDV encephalomyelitis, have high levels of CDV antibodies in their cerebral spinal fluid, indicating an active local immune response (Johnson et al., 1988). These antibodies have been shown to be directed solely to the M protein of CDV in 2/5 cases, which is the pattern observed in the homologous measles virus-induced SPE of humans; the remaining dogs demonstrated antibodies to nucleocapsid and phosoprotein antigens as well (Rima et al., 1987).

5.2.8. Chronic active hepatitis

Six types of chronic hepatitis have been described in dogs:

1. chronic active hepatitis;
2. chronic persistent hepatitis;

3. chronic cholestatic hepatitis;
4. fibrosing hepatitis with cirrhosis;
5. chronic cholangiohepatitis; and
6. miscellaneous secondary hepatitis (Fuentealba et al., 1997).

About one-third of all of these cases are of the chronic active type. Chronic active hepatitis occurs most commonly in Doberman pinschers and is characterized by inflammation and scar tissue formation around branches of small hepatic veins and apoptosis of hepatocytes in zone 3 (Thornburg, 1998). The inflammatory reaction consists predominantly of macrophages, lymphocytes, and plasma cells, indicating a type IV immune reaction. The exact etiology of this condition is unknown, but the strong breed predisposition tends to support autoimmunity. However, autoimmunity was suspected to be the cause of virtually all chronic active hepatitis in people before hepatitis B, C, D and G virus infections and a number of therapeutic drugs were a demonstrated as causal agents (Desmet, 1997).

The disease in Dobermans is more common in females and the usual age of onset of signs is 2–8 years. However, biopsy studies indicate that actual lesions may be present for up to five years before the condition becomes clinical (Speeti et al., 1998). Clinical signs include weight loss, atrophy and other irregularities of the liver parenchyma on ultrasonography, elevated liver enzymes, and sometimes jaundice. Corticosteroid therapy may be beneficial to some affected dogs (Dill-Macky, 1995).

6. Paraproteinemias (Dysproteinemias)

Paraproteinemias are characterized by pronounced elevations of normal or abnormal immunoglobulins in the blood.

6.1. Polyclonal gammopathies

Polyclonal gammopathies are broad based elevations in all immunoglobulin classes. They are idiopathic, associated with infectious of immunologic diseases, or a consequence of aging.

There is a tendency for immunoglobulin levels to rise with age. Old animals and humans generally have higher immunoglobulin levels, especially IgG, than younger animals. Elevations in immunoglobulins with aging are often associated with an increased incidence of autoantibodies and decreasing levels of normal cellular immunity.

6.2. Monoclonal gammopathies

Monoclonal gammopathies are characterized by elevations in specific immunoglobulin classes, usually derived from a single clone of B-cells. The pathologic immunoglobulin is usually not directed at any identifiable antigen. In rare cases, however, the antibody specificity is identifiable (usually because of some pathologic effect of the monoclonal antibody). Monoclonal immunoglobulins are also called M-proteins. Monoclonal

gammopathies are either benign or malignant, with the latter being most common in the dog.

6.2.1. Monoclonal gammopathies associated with chronic infectious diseases

The line between polyclonal and monoclonal gammopathies can be blurred in certain chronic infectious diseases. Fourteen dogs with chronic *Ehrlichia canis* infection were found to have gammopathy; serum protein electrophoresis demonstrated patterns of distinct narrow based monoclonal spikes, broad based monoclonal spikes, and monoclonal spikes superimposed on polyclonal spikes (Breitschwerdt et al., 1987). A monoclonal gammopathy causing hyperviscosity and acute blindness has also been seen in a dog with ehrlichiosis (Harrus et al., 1998). A German shepherd dog with ehrlichiosis and monoclonal gammopathy was treated with both antibiotics and plasmapheresis (Matus et al., 1987). A monoclonal gammopathy has also been observed in a dog with plasmacytic gasteroenteritis; the gammopathy resolved with dietary and immunosuppressive treatment (Diehl et al., 1992).

6.2.2. Multiple myeloma

A multiple myeloma is a malignancy derived from a single plasma cell that has been clonally expanded. Myelomas can produce no immunoglobulins or parts of immunoglobulins (MacEwen et al., 1984; Marks et al., 1995), whole immunoglobulins of any class (Couto et al., 1984; Finnie and Wilks, 1982; Kirschner et al., 1988; Orr et al., 1981), or parts of immunoglobulins such as light or heavy chains (Hoenig, 1987). Myelomas occur almost exclusively in aged animals. Myelomas in dogs are recognized more frequently in Doberman Pinschers (Hoenig, 1987) than other breeds. Myelomas in dogs are about 50% IgA, 45% IgG, and 5% IgM.

6.2.3. B-cell lymphoma

A small percentage of lymphoblastoid tumors of B-cell origin (usually CLL) secrete M-protein (Breuer et al., 1993). The most common M-protein associated with B-cell lymphosarcomas is IgM, followed by IgG. IgA is usually not secreted by lymphoblastoid cell tumors. Features of lymphosarcomas that secrete M-proteins are similar to those described for multiple myelomas.

6.2.4. Disease syndromes associated with monoclonal gammopathies

Clinical signs are referable to:

1. space occupying lesions, usually in flat bones such as skull, vertebrae, ribs, or pelvis, which cause pain, limping, diarrhea, neurological signs, etc. (Gibson et al., 1997; Maeda et al., 1993; Raskin and Krehbiel, 1988; Tuch and Tuch, 1992), or in soft tissues such as liver, spleen, intestine and bone marrow (Kato et al., 1995; Peterson and Meininger, 1997);
2. hyperviscosity syndrome, usually associated with IgA or IgM myelomas (Center and Smith, 1982; Kirschner et al., 1988);
3. cryoglobulinemia (abnormal protein that precipitates when cooled);
4. paraneoplastic syndrome (Hendrix et al., 1998; Villiers and Dobson, 1998);

5. amyloidosis associated with catabolism of immunoglobulins (Geisel et al., 1990; Platz et al., 1997); or
6. immunosuppression (normal immunoglobulins are greatly depressed in animals with monoclonal gammopathies).

Myelomas in dogs are equally distributed between the skeleton, usually flat bones, and soft tissue organs such as the intestinal tract, spleen, and other internal organs. Plasma cell leukemias are rare (Couto et al., 1984). Bone lesions are often lyric in nature and if they are in areas such as vertebral bodies, clinical signs such as paresis may result. Tumor involvement of internal organs is more likely to be associated with vague signs of disease and weight loss occurring over many weeks or months.

Hyperviscosity syndrome is a specific complication of certain monoclonal gammopathies. IgM or IgA M-proteins, if present in high enough concentration, will greatly increase the viscosity of the serum. Hyperviscosity will lead, in turn, to an increased load on the heart and hypertension, and vascular thrombosis due to blood sludging in smaller vessels. Animals with hyperviscosity syndrome commonly present with signs of heart failure, collapse, retinal hemorrhages, or acute neurologic signs.

Some M-proteins, usually of the IgM class, behave as cryoglobulins and will precipitate in smaller blood vessels when the serum is cooled. Cryoglobulinemia is usually associated with gangrene of the extremities (ear tips, digits, eyelids, tip of tail, etc.). Exposure to cold weather may exacerbate or precipitate clinical signs.

Pathogenic function of M-protein may be related to specific antibody binding to host antigen. Some M-proteins behave as cold agglutinins and animals will present with signs referable to hemolytic anemia and thrombosis. A human with a bizarre neurologic syndrome was found to have M-proteins specific for a nerve tissue antigen. A similar situation may have occurred in a German shepherd dog with multiple myeloma and associated polyneuropathy; the neuropathy disappeared when the dog was given chemotherapy for the tumor and the gammopathy resolved (Villiers and Dobson, 1998).

Amyloidosis is a common and sometimes fatal sequelae of idiopathic monoclonal gammopathies, or immunoglobulin-secreting malignancies, in man. The relationship of amyloidosis and monoclonal gammopathies is not as obvious in animals, probably because animals have a much lower incidence of idiopathic monoclonal gammopathy than man and are therefore more apt to present with more common signs of the tumor.

Immunosuppression (hypogammaglobulinemia) is frequently associated with monoclonal gammopathies in people. People with gammopathies have abnormally low levels of normal immunoglobulin and suffer from chronic skin, respiratory, and intestinal tract infections. Immunosuppression, however, is not usually associated with monoclonal gammopathies of animals. Once again, this is probably due to the low incidence of idiopathic monoclonal gammopathies in animals compared to man.

6.2.5. Diagnosis of gammopathies

Total protein levels are usually elevated in animals with gammopathies, and this may be the first tip-off of the condition. Many conditions falsely raise the total protein levels measured by refractometer, e.g. hyperbilirubinemia, hyperlipidemia, hyperfibrinogenemia, hemolysis, and dehydration. The total protein and albumin levels measured on

automated chemistry panels is probably a better indication of globin increases (TP − albumin = globulin).

Serum electrophoresis gives a relative measurement of the concentrations of major proteins in the serum. Monoclonal proteins may show up as specific M-protein spike in the electrophoresis profile. Serum levels of IgG, IgA, and IgM can be measured by a radial-immunodiffusion assay. Polyclonal gammopathies will have elevations of all Ig classes, while monoclonal gammopathies will have an elevation of one Ig class and depressions of the others.

Serum can also be tested for hyperviscosity. Serum is allowed to drip through a pipette tip or needle. The time required for a given volume of normal serum to pass through the needle or pipette is compared with the time required for the abnormal serum. The test can also be run by comparing the time an abnormal and normal drop of serum takes to run down to the bottom of an inclined glass plate.

Cryoglobulins are usually picked up by technicians doing the automated blood chemistries. The precipitate in the cooled serum will usually plug the tip of the sample aspirator. Serum can be cooled and centrifuged; a dense precipitate will sediment to the bottom of the tube. The precipitate will dissolve when warmed to 37°C.

A common test for monoclonal gammopathies in humans is the Bence–Jones protein test on urine. Bence–Jones protein is mainly light chains of immunoglobulin. Excess light chain secretion into the urine can occur as a result of catabolism of large amounts M-protein or originate from myelomas that produce free light chains rather than intact immunoglobulins. Urine is treated with NH_4SO_4, and a precipitate indicates presence of Bence–Jones protein. Unfortunately, the Bence–Jones protein test is not very diagnostic in dogs, because very few myelomas in dogs produce free light chains.

6.2.6. Treatment of paraproteinemias

The treatment of polyclonal gammopathies is never directed at the production of the immunoglobulin per se, but rather at the inciting cause.

Multiple myelomas are treated with prednisolone and alkylating agents such as Alkeran (phenylalanine mustard). Prognosis is good for remission in dogs, but the long-term prognosis is poor due to relapse. Specific lymphoma chemotherapy protocols are used to treat monoclonal antibody producing pre-B-cell tumors.

If hyperviscosity is a problem, then plasmapheresis or removal of plasma is indicated. The same treatment may be necessary to lower the levels of monoclonal cold agglutinins.

7. Immunodeficiency diseases

7.1. Review of innate and adaptive immunity

The following discussions are based on reviews of the immune system in dogs, humans and other species (Felsburg, 1994; Janeway and Travers, 1996).

7.1.1. Innate immunity

Immunity is both innate and adaptive in nature. Innate immune mechanisms are non-specific, require no previous experience with the invading microbe, and are immediately

available. Innate immunity is effective against most microorganisms encountered daily in life and that cause perceptible disease only on occasion. In contrast, adaptive immunity occurs only when microbes breach innate defenses; such microbes tend to be highly antigen specific and require several days to reach maximum growth. Adaptive immune responses are characterized by the generation of antigen-specific cells (B- and T-lymphocytes) that specifically target the pathogen, as well as memory cells that allow rapid response to reinfection.

Innate immunity appeared very early in evolution and is the sole defense mechanism in lower animals. Various components of the innate immune system served as 'evolutionary scaffolds' for subsequent adaptive immune responses. This step-wise evolution is recapitulated during an immune response; innate immunity slows down the infection, thus allowing time for adaptive immunity to take hold, and provides both the trigger and 'scaffolding' on which adaptive immune reactions develop.

Epithelial and mucosal barriers are the first lines of defense. The mucosa is thrown up into microvilli to increase surface area and to retain the mucus layer. Air, in the case of the respiratory tract, or digesta, in the case of the intestinal tract, move over this surface film. The mucous film contains phagocytes and a number of non-specific antimicrobial substances. The cornified layer of skin seals off and protects living cells underneath and acts as a sponge for normal flora and other non-specific protective substances. Non-specific antimicrobial substances include fatty acids (skin), lysozyme (saliva, sweat, tears), pepsin (gut), hydrochloric acid (stomach), and antibacterial peptides such as the defensins (intestine). Normal skin and mucosal flora also compete for nutrients and attachment to epithelium, and produces antimicrobial substances.

The alternative pathway of complement activation is another arm of the innate immune response. C3 is constantly being converted (activated) to C3b at low levels. C3b is attracted to cell membranes, where it activates and combines with another serum protein called Bb, forming C3b/Bb complexes. Additional proteins called CR1, MCP and DAF are present on the surface of normal cells and act to inactivate C3b/Bb complex. C3b/Bb is a C3 convertase that leads to conversion of C3 to C3b. The surfaces of many microbes lack these enzymes, hence cell bound C3b/Bb is not inactivated. C3/Bb can act as an opsonin for phagocytes and as an activator of the complement cascade.

The properdin system is another potent non-specific defense mechanism. Many microbes favor the binding of properdin (factor P). Factor P stabilizes the C3/Bb complex, thus preventing their dissociation and allowing the buildup of C3b and activation of the classical complement pathway.

Macrophages also play a key role in innate immunity and are the triggering cells for many adaptive immune responses. Macrophages mature continuously from circulating monocytes, which leave the circulation and migrate into tissues throughout the body. Macrophages possess a number of surface receptors for microbes, including mannose receptors, scavenger receptor, bacterial lipopolysaccharide binding receptor, and leukocyte integrin (CR3 or Mac-1). These receptors can bind with surface proteins on a wide range of microbial pathogens, thus triggering phagocytosis and secretion of cytokines such as IL-1, IL-8, TNF-α, IL-6 and IL-12.

Cytokines are key substances in the development of adaptive immunity. They induce local inflammation and attract inflammatory cells into the area through increased

production of adhesion molecules on the vascular endothelium. These inflammatory cells include neutrophils, eosinophils, lymphocytes and monocytes. Increased vascular permeability also results, leading to an influx of fluids.

Microphages, i.e., neutrophils, are abundant in bloodstream but are not normally found outside of blood vessels in normal circumstances. The innate immune response produces many substances that are chemotactic for neutrophils. Bacterial cell wall components can bind directly to membrane receptors on neutrophils. Lipopolysaccharide binding protein in serum can bind bacterial LPS and the complex can be bound to neutrophils. Bacteria phagocytosed by neutrophils can be killed by toxic oxygen metabolites, proteases, phospolipases and defensins.

Acute phase reactants also function in the innate immune system. Certain proteins are produced in response to the release of cytokines such as TNF-α, IL-1 and IL-6. C-reactive protein consists of five identical subunits, which binds to phosphoryl chloline in the cell walls of bacteria and fungi. C-reactive protein binding to microbial cell wall induces opsonization and activates the complement cascade. Mannose binding protein can also react with mannose residues on the surfaces of many microbes and induce phagocytosis and complement activation.

Interferons are small proteins that are produced by virus infected cells. Interferons are produced by many types of virus infected cells and act on distant cells to render them resistant to viral replication. Interferons α and β are induced during the innate phase of immunity, while interferon γ appears only during the adaptive stage of the immune response. Interferons also activate natural killer (NK) cells and upregulate MHC class I receptor expression. The latter activity protects normal cells from non-specific destruction by NK cells.

Natural killer cells are a population of T-lymphocytes that lack CD8 and CD4 surface markers. NK cells evolved much earlier in phylogeny than T- and B-cells responsible for adaptive immunity. NK cells become potent killers when they receive specific activation signals. NK cell activity is enhanced $100\times$ by interferon α, β and IL-12. Therefore, invasion of host cells by most microbial pathogens will activate NK cells.

Activated NK cells can bind to infected cells by several mechanisms. Infected cells elaborate increased levels of sulfated proteoglycans on their surfaces. NK cells have receptors that will bind to this substance, and NK cell binding leads to the elaboration of cytotoxins that act at very short range. The activation of NK cells to killer cells is inhibited, however, if the NK cell identifies self-MHC-I proteins; hence the importance constant MHC-I upregulation in normal cells. Virus infected cells will elaborate fewer MHC-1 receptors, either by viral interference with protein synthesis or virus blocking of MHC-1 export to the cell surface. Such cells are resistant to interferon α and β induced MHC-1 upregulation. Host cells that do not display MHC-I will then be killed by activated NK cells. There is also evidence that virus infected cell may introduce new peptides into the MHC receptors, thus preventing normal self-recognition by NK cells.

T-cells bearing the γ and δ cell surface proteins are found specifically in epithelial surfaces (skin and mucosa). They do not recirculate and their surface receptors are homogeneous in any one epithelium. Because of the homogeneity of their receptors, it is thought that $\gamma : \delta$ T-cells do not recognize specific microbes, but rather respond to non-specific changes induced in a particular epithelium as a result of some insult (usually

microbial). $\gamma : \delta$ T-cells can recognize epithelial cells that have been affected by stress or heat shock proteins, as well as cells that have aberrant expression of self-recognition (i.e., MHC) markers. Intra-epithelial $\gamma : \delta$ T-cells possess killer activity following receptor binding to other cells.

Innate immunity also involves a certain subpopulation of B-cells. The production of specific antibodies by conventional B-cells is important in adaptive immunity. There is, however, a separate lineage of B-cells (i.e., CD5+) that are more limited in their V gene diversity, that are self-renewing without previous exposure to antigen and T-cell independent, and that predominate in certain areas of the body such as the peritoneal cavity. They do not undergo immunoglobulin class switching and their predominant immunoglobulin production is IgM. CD5+ B-cells do not exhibit immunologic memory, and antibody production peaks by 48 h, which is much faster than regular B-cells undergoing an adaptive immune response. CD5+ B-cells make antibodies mainly to polysaccharide antigens present on many bacterial cell walls. It is thought that CD5+ B-cells are evolutionary precursors of conventional B-cells, just as $\gamma : \delta$ T-cells may be precursors of $\alpha : \beta$ T-cells. The 'natural' antibodies produced by CD5+ B-cells probably play an important role in natural defenses against bacterial infection, especially within the peritoneal cavity.

7.1.2. Adaptive immunity

T-cells bearing the $\alpha : \beta$ surface markers are central to adaptive immunity, and were preceded in evolution by $\gamma : \delta$ T-cells. The first step in adaptive immunity is antigen recognition and T-cell activation. T-cell activation occurs when antigen (free or cell-associated) is encountered in draining lymphoid organs. Antigen in the form of particulate substances or whole microbes is taken up by macrophages and antigen presenting cells. These cells present the specific antigenic signal on their surfaces either in context of MHC-I (cell-associated microbes) or MHC-II (ingested particulate antigen).

Activation of the innate immune system leads to monokine release and increased trafficking of CD4+ T lymphocytes through the involved lymphoid organs. This increased trafficking of naive CD4+ T-cells increases the likelihood that a cell bearing the appropriate receptor will be brought into contact with its corresponding antigen.

T-cell differentiation is central to the direction of the adaptive immune response. When naive CD4+ T-cells contact their specific antigen, a process of differentiation is triggered. This differentiation can follow two general paths: (1) the cell can become an inflammatory (effector) T-cells, i.e., T-helper 1 cells or Th1-cells, or (2) a helper T-cells, i.e., T-helper two cells or Th2-cells. The course that most cells take during an immune response depends on the cytokine milieu generated during the innate phase of immunity. Generally speaking, Th1-cells generate cell-mediated immunity, while Th2-cells induce antibody production. CD8+ T-cells are activated in the same manner and are destined to become specific cell killers (i.e., cytotoxic T-cells).

T-effector cells are fully activated by 4–5 days into an infection. They are guided to the specific site of infection by upregulated surface markers (integrins) on their surfaces and corresponding upregulated receptors (cell adhesion molecules, selectins) on the surfaces of endothelial cells in the target area. Inflammatory T-cells activate macrophages at the

site of infection, which greatly enhances their ability to kill intracellular microbes. CD8+ cytotoxic cells act in a manner similar to NK cells in the killing of infected cells.

Activated T-effector cells attach to infected target cells through the double key mechanism of (1) identifying the specific antigen arrayed within the MHC and (2) recognizing self-markers also in the MHC (see discussion in section on Innate Immunity). When the proper recognition signals are received, the T-effector cells will elaborate cytotoxins that will kill the abnormal cell.

Naive B-cells possessing the appropriate surface receptor cannot be activated until they contact an armed T-helper cell that is specific for one of the peptides derived from that antigen. Specifically stimulated B-cells undergo rapid clonal expansion and differentiation, going from mature resting B-cells, to plasmablasts, and finally to plasma cells. Initial antibody production is usually of the IgM type and occurs by lymphoblasts in the T-cell areas of the lymphoid organs. Plasmablasts migrate to bone marrow and medullary cords where they become sessile plasma cells. Plasma cells are highly efficient at antibody production, undergo class switching (IgM to IgG to IgA), and can sustain low level antibody production from prolonged periods of time.

IgM is a pentameric form of IgG and was the first immunoglobulin class to evolve (in lower fish). Because ontogeny recapitulates phylogeny, IgM is also the first antibody system to develop in a fetus and is the first class of antibody to appear during the evolution of an individual immune response. For instance, new born puppies can produce IgM upon antigenic stimuli late in gestation and at birth, while IgG and IgA responses take several weeks longer to manifest (2–4 weeks of age). If an older puppy is infected with a microbe, the first antibodies that appear are of the IgM class, followed later by IgG and IgA.

IgM is the most primitive and important immunoglobulin class. IgM antibodies are strong complement binders, strongly lyric, and circulate both in the plasma and are secreted unto mucous membranes. Therefore, IgM shares many of the functions found collectively in other Ig subclasses. IgG makes up most of the circulating antibodies. Unlike IgM, it can gain access to the interstitial fluids. It is the predominant antibody made in lymph nodes and spleen and has major importance against systemic microbial infections. IgA is a dimer and evolved after IgM and IgG. It is produced in greatest amounts in diffuse lymphoid aggregates and is the principal secretory antibody. The IgA system has very little immunological memory and depends on continuous antigenic exposure for maintenance. IgA is important for control of local infections of mucous membranes. IgE is a monomer and is the most recently evolved Ig. Like IgA, IgE is secreted mainly by plasma cells present in diffuse lymphoid aggregates underlying skin and mucous membranes. IgE is non-complement binding and is intimately associated with circulating basophils and tissue mast cells. IgE binds with great avidity to cell membrane receptors present on these cells. IgE and its associated mast-cell responses are intimately associated with parasite immunity.

Although antibody binding provides specificity, the ultimate effect of that binding is usually determined by complement. The complement system is comprised of large number of distinct plasma proteins. When the initial complement components are appropriately triggered on the surface of the pathogen, either by antibody-, lectin/mannose-, or alternative-pathways, a cascade of reactions occurs with preceding proteins

acting as enzyme activators for subsequent proteins. Earlier components of the cascade (C3a, C4a, C5a), act to recruit phagocytes to the site or to enhance opsonization (C3b), while terminal complement components (C5b, C6, C7, C8, C9) lead to microbial lysis. In the classical pathway, C1q molecule binds to antibody molecule to trigger buildup of C3 convertase on pathogen surface through activation of C1, C4, and C2. The complement cascade can also be activated through the lectin pathway, where mannose-binding protein binds mannan on pathogen surface leading to buildup of C3 convertase through MBP : MASP, C4 and C2. A third, or alternative, pathway of complement activation involves the buildup of C3, proteins B and D on the pathogen's surface and leading to C3 convertase.

One outcome of adaptive immunity is the establishment of immunologic memory. Memory is the ability of the immune response to respond more rapidly and efficiently to pathogens that have been encountered previously. Immunologic memory relies on both the clonal expansion and clonal differentiation of specific memory lymphocytes during the primary immune response. Memory T-cells are long-lived. Retained antigens, either particulate or organismal, may be important in maintenance of memory.

7.2. Immunodeficiency states

The characteristic features of immunodeficiency:

1. infections caused by microbes that are normally not pathogenic;
2. unusually severe or persistent infections that are normally mild and self-limiting;
3. an inordinate number of different infections in the same individual;
4. recurrence of infections that occurred in a subclinical form earlier in life, and
5. infections that are unexplainably difficult to treat.

Immunodeficiency states involve either innate or adaptive immune responses and are of congenital or acquired origin.

7.2.1. Congenital immunodeficiency

7.2.1.1. Congenital defects in innate immunity. Congenital causes of innate immunodeficiency are varied and common in humans and are being increasingly recognized in dogs. Although very little clinical research has been on dogs in regards to deficiencies in more classical aspects of innate immunity, there are several areas where such defects are obvious.

Normal anatomy is essential for the integrity of the individual, but few people think of immunity when they study anatomy. However, anatomic defects are probably the most common cause of innate immunodeficiency related illnesses in dogs. Tissues that cover both external and internal body surfaces are the most important component of innate immunity. Skin has an outer cornified layer that resists microbial growth and invasion. Glandular secretions onto the skin and mucous membranes also contain non-specific antimicrobial substances. Some Sharpei dogs have abnormal skin and are predisposed to constant skin infections. The cilia of the mucous membranes, especially those of the

respiratory and genitourinary tracts, act as brooms to trap and sweep out foreign material. The ciliary layer also acts as a substrate to trap and hold mucus secretions. Congenital defects of cilia, which render them immobile, occur in humans and animals. Dogs with immobile cilia syndrome (primary ciliary dyskinesia) suffer greatly from recurrent and difficult to treat infections of the upper and lower airways (Edwards et al., 1992).

Small brachycephalic breeds of dogs suffer much more from periodontal disease than larger sporting breeds. These small breeds often have malpositioned jaws and teeth, which alters the pressure that is normally placed on the periodontial ligaments. Many small breeds will not chew on hard objects, which further decreases the exercise of the periodontial tissues and prevents tarter removal. The net effect of these abnormal forces is a gradual weakening of the periodontial ligaments, tooth mobility, periodontial pocket formation, bacterial over colonization, periodontitis, resultant local bone resorption, and more tooth instability.

A wide range of anatomic anomalies of tissues other than those of skin and mucous membranes have been associated with immunodeficiency. For instance, the sphincters of the urogenital, gastrointestinal and respiratory tracts act as doors to prevent the influx of microbes. Animals with congenital anomalies of the urethral sphincter or ectopic ureter will leak urine continuously to the outside. Bacteria then grow up the urine stream and infect the bladder. Animals with abnormal ureters that obstruct normal urine flow from the kidney often develop hydroureter and hydronephrosis; such animals are very prone to severe and recurrent bacterial pyelonephritis. Animals with congenital anomalies of the larynx may aspirate food into their lungs and develop pneumonia. Individuals with cleft hard or soft palates will frequently develop chronic nasal infections due to foreign material being drawn into the nasal cavity. Certain breeds of dogs flattened faces and protruding eyes may have problems with tear drainage, causing excessive wetness around the eyes and bacterial overgrowth. A similar situation occurs in animals with congenital blockage of the lacrimal ducts. Dogs are much more prone to external ear infections than other species because of the length and angulation of their external ear canals. Dogs that have hair growing down their ear canals are even more prone to infection than dogs that do not. Substances secreted onto the skin and mucous membranes by the various glands have an important non-specific microbial function. Dogs that secrete too little substances onto the skin are much more prone to chronic and recurrent skin infections. Paradoxically, too much secretion can have the same effect. Therefore, animals with both seborrhea sicca and seborrhea oleosa suffer from chronic skin infections.

7.2.1.2. Congenital defects in adaptive immunity. Congenital anomalies of virtually all aspects of adaptive immunity have occurred in people and animals. Most of the literature on these anomalies has been generated from naturally occurring disease in humans or inbred strains of laboratory mice.

Congenital defects in phagocytosis range from mild to highly lethal, depending on their nature and severity. Because severe defects usually lead to early death, it is the less severe phagocytic defects that are often seen clinically. A defect in leukocyte adhesion molecules CD11b and CD18 on neutrophils occurs as an X-linked recessive trait in Irish Setters (Trowald-Wigh et al., 1992). Affected animals suffer from recurrent fevers, polyarthritis, skin lesions, and stunted growth almost from birth and seldom live past one

year of age. They have extremely high levels of mature hypersegmented neutrophils in their blood. Neutrophils are being summoned in large numbers from the bone marrow, but few of them find their way into sites of inflammation. Poorly characterized neutrophil phagocyte abnormalities have been reported in Weimeraners and associated with recurrent infections (Hansen et al., 1995).

Cyclic neutropenia in grey Collies—Some grey Collie dogs, and rare mongrels with similar coat color, have profound cyclical fluctuations, usually over a 12–14 day period, in the numbers of neutrophils (Yang, 1987). The disorder is due to an autosomally recessive gene and is identical to human cyclic neutropenia (Yang, 1987). Periods of bone marrow inactivity and neutropenia are followed by a period of flamboyant bone marrow activity characterized by monocytosis, thrombocytosis, reticulocytosis and neutrophilia (Pratt et al., 1990). Affected dogs are very prone to local and systemic bacterial infections, often of a cyclic nature; the pathological effects are cumulative and affected puppies rarely survive to six months of age (Yang, 1987).

The disorder is due to a heritable recessive regulatory defect of hematopoietic stem cells (Dale et al., 1995). An abnormal response to granulocyte colony stimulating factor (G-CSF) has been implicated in the pathophysiology of the syndrome, in particular a defect in the G-CSF signal transduction pathway distal to G-CSF receptor expression or binding (Avalos et al., 1994). The neutropenia has greatly improved with G-CSF treatment, although cycling was still observed (Lothrop et al., 1988; Mishu et al., 1992). Better results may have been obtained when G-CSF was given in combination with stem cell factor (Dale et al., 1995).

Complement deficiencies involving a number of the individual components of the classical and alternative complement proteins have been described in man and various animal species. These abnormalities involve either the total absence of a complement factor or the production of a factor that is structurally and functionally abnormal. Dogs with complement deficiencies often live into adulthood and beyond, but tend to suffer from atypical, recurrent, or persistent infections of the skin and respiratory tract (Ameratunga et al., 1998; Blum et al., 1985). Humans with complement deficiencies also have a higher incidence of some immunologic diseases such as SLE and rheumatoid-like arthritis (Sleasman, 1996).

Heritable *deficiencies of humoral immunity* are surprisingly common. They usually involve deficiencies of specific immunoglobulin classes. The most common heritable deficiency of this type in man and animals involves IgA. About 1 : 500 people lack IgA. Absolute deficiencies of IgA are common in Chinese Shar peis, while partial deficiencies are common in German shepherd dogs (Day and Penhale, 1988a). The disorder has also been observed in other breeds, such as the Beagle. IgA deficiencies are manifested by increased incidence of skin and respiratory infections (Felsburg et al., 1985). Individuals with IgA deficiencies often have more problems with allergies, probably because the IgE system is called upon to compensate for the lack of IgA-mediated immunity. Several bloodlines in a colony of English cocker spaniels were reported to have relative IgA deficiency, a high incidence of ANA, Coomb's antibodies, SLE, hypothyroidism and idiopathic cardiomyopathy (Day, 1996a, b, c). Attempts to link aspergillosis in German shepherd dogs to their variable IgA deficiency have been negative (Day and Penhale, 1988a).

Some German shepherd dogs appear to have a selective inability to mount normal humoral immune responses. The majority of dogs with systemic aspergillosis, caused by *A. terreus*, do not develop demonstrable antibody responses to the organism by agar gel diffusion, counter immunoelectrophoresis, ELISA and indirect immunofluorescence (Day and Penhale, 1988b). However, these same animals have significantly increased polyclonal IgG production.

Severe combined immunodeficiencies, commonly known as SCIDs, occur in both man and animals and is usually associated with heritable defects in metabolic pathways critical for T-cell development. The classical form of this SCIDs is the 'boy in the glass bubble', associated with adenine deaminase deficiency. A similar disease, but with a different genetic basis, occurs in Basset hounds. Affected Bassett hounds often develop canine distemper at 10–16 weeks of age, when their maternal immunity wane. The source of the distemper virus is from their live virus vaccines. Animals that do not develop distemper, will usually fail to thrive and die by 5–6 months of age. SCID, associated with a X-linked defect in interleukin-2 (IL-2) receptor function, was described in a family of Cardigan Welsch corgis (Deschenes et al., 1994; Jezyk et al., 1989; Pullen et al., 1997). The defect is caused by a single nucleotide insertion in the γ chain of the canine IL-2 receptor (Somberg et al., 1995). The same defect has been observed in a subset of people with SCIDS (Henthorn et al., 1994). Clinical features include failure to thrive, hypogammaglobulinemia, absent T-cell mitogen responses, and thymic dysplasia and lymphoid hypoplasia (Snyder et al., 1993; Somberg et al., 1996). Dogs raised in conventional environments usually die of environmental or vaccine-virus associated infections by five months of age. Dogs with the defect can be maintained indefinitely in gnotobiotic environments, although the incidence of leukemias may be increased (Felsburg et al., 1994).

Selective immunodeficiencies of unknown type have been well described in dogs, virtually always in purebreds. German shepherd dogs are known to be much more susceptible to *Ehrlichia canis* infection than mongrels and Beagles (Nyindo et al., 1980), to nasal (*Aspergilla fumigatus*) and systemic aspergillosis (*A. terreus*) (Berry and Leisewitz, 1996; Day and Penhale, 1988a, b; Day et al., 1985, 1986; Kabay et al., 1985; Mortellaro et al., 1989), and to generalized pyodermas (Rosser, 1997). Rottweilers are particularly susceptible to parvovirus infection, nasal and systemic fungal infections, and oral papillomavirus infection (Bredal et al., 1996). Certain Dachshunds have developed severe *Pneumocystis carinii* infections of the lungs (Lobetti et al., 1996), a disorder only described in human AIDS patients. A certain subset of young (Medleau and Willemse, 1995), and occasionally aged (Duclos et al., 1994), dogs appear to be more susceptible to systemic demodectic mange. Great Danes and Doberman pinchers are more susceptible to cryptococcal infections than other breeds (Malik et al., 1995). Labrador and Golden retrievers are 5–6 times more susceptible to a type of glomerulonephritis that has been putatively associated with *Borrelia burgdorferi* infection (Dambach et al., 1997). *Neospora caninum* infection, in both neonatal and adult forms, is almost always observed in purebred dogs (Barber and Trees, 1996; Cuddon et al., 1992; Flagstad et al., 1995; Jacobson and Jardine, 1993; Poli et al., 1998; Pumarola et al., 1996). Dogs with selective immunodeficiencies have normal immune responses to other infectious agents, normal complement and Ig levels, and will even respond favorably if treated properly for the problem infection (Day et al., 1985).

Uncharacterized immunodeficiencies have been reported in several breeds. An immunodeficiency syndrome has been described in Irish Setters (Cauvin and Connolly, 1997).

An intriguing syndrome manifested by acute fever, gastrointestinal signs, and malaise has been observed in Weimeraner puppies (Couto et al., 1989). These initial signs, which vary in severity, are often followed by mild to severe hypertrophic osteodystrophy (HOD) (Woodard, 1982). In some animals, recurrent disease signs involving the alimentary tract, joints, skin, peripheral lymph nodes, central nervous system and conjunctiva persist through adolescence (Day et al., 1997). The condition has been reportedly associated with variable decreases in one or more immunoglobulin isotopes (Couto et al., 1989; Day et al., 1997) and possible neutrophil functional abnormalities (Couto et al., 1989; Hansen et al., 1995). Although most breeders feel that the condition is triggered by vaccination (Dodds, 1999), some puppies have developed the condition even when not vaccinated. The condition appears to fall in the category of common variable immunodeficiency (CVID) of humans. CVID is a heterogenous syndrome (Spickett et al., 1997), characterized by humoral immunodeficiency involving one or more immunoglobulin isotypes, recurrent bacterial infections and a variety of other immunologic abnormalities (Eibl and Wolf, 1995; Eisenstein and Sneller, 1994). Affected humans show various defects in T-cell regulation of B-cells, B-cell activation, or of cytokine responses. However, there are notable differences in the Weimaraner syndrome from CVID in people:

1. The disease occurs solely in puppies, whereas CVID can be seen in infants, children and adults;
2. CVID is rarely self-limiting, whereas many affected dogs appear to outgrow the condition; and
3. affected dogs respond dramatically to steroid therapy, while humans are treated with γ globulin to compensate for the antibody deficiencies.

If inappropriately treated, many affected Weimaraner puppies will die or be euthanitized. Glucocorticoid treatment is highly effective in breaking the febrile response, resolving systemic signs, and hastening the resolution of the HOD. Unfortunately, many veterinarians will not use this treatment, because they mistakenly view the condition purely as an immunodeficiency.

A suspected primary immunodeficiency syndrome has been seen in three related Irish wolfhounds (Leisewitz et al., 1997). The dogs presented at less than one year of age with a history of chronic nasal discharge and persistent lower respiratory tract disease. The signs improved on antibiotic treatment, but recurred when treatment was stopped. A cell-mediated immunodeficiency was suspected based on cursory testing, but this diagnosis is not compatible with this type of clinical signs. IgA or complement deficiencies, and cilliary dyskinesis, would be more likely causes.

A primary immunodeficiency syndrome may also occur in Chinese Shar-pei dogs (Rivas et al., 1995). The presenting signs were recurrent infections and malignancies. Abnormally low levels of serum IgM and IgA were present in most affected dogs, and low IgG in a lesser proportion.

7.2.2. Acquired immunodeficiency

7.2.2.1. Innate immunity. Diseases that compromise the integrity of the epidermis and overlying cornified layer can be complicated by secondary infections with resident bacteria, especially *S. aureus*. Burns, atopic dermatitis, flea bite dermatitis, and bullous diseases are just a few examples of primary skin diseases that are often complicated by secondary infections. Normal flora in the skin, oropharynx, and lower GI tract prevent the establishment of abnormal flora. If the normal flora is compromised, pathological flora can take selective advantage of the situation. This problem is particularly serious with antibiotics are used in the presence of antibiotic resistant microorganisms.

Many surgical procedures require the use of endotracheal tubes, forced respiration, and inhalant anesthetic gases. Endotracheal tubes will effectively bypass the innate immunity provided by the upper airways and carry oral bacteria down to the mainstem bronchi. Forced respiration during anesthesia can drive secretions from the upper air passages to the lower airways. This is a serious problem when there is already preexisting lung disease.

Indwelling urinary catheters are a major source of infection for the urinary tract, and IV catheters a major source of infection for the blood. Urethrostomies frequently lead to cystitis, tracheostomies to tracheobronchitis, and oronasal fistulas to rhinitis.

Acquired anomalies of phagocytosis are usually due to conditions that markedly decrease the *number* of available phagocytes. In contract, congenital phagocyte anomalies are usually associated with a decrease in the function of the phagocytes. In either case, the net effect is a diminished phagocytic capacity. This is usually manifested by an increased incidence of infections caused by bacteria. Anticancer drug therapy often leads to profound cytopenias during the induction stages of treatment, putting them at grave risk for infection. Autoimmune and drug induced neutropenias of immune origin are often manifested by fevers and sepsis. Ninety percent of dogs necropsied for acute parvovirus infection had recoverable *E. coli* in their lungs and liver (Turk et al., 1990); secondary infections with *Candida albicans* (Rodraiguez et al., 1998), *Clostridium perfringens* (Turk et al., 1992), *Edwardsiella tarda* (Van Assche, 1991) have also been described. These secondary infections occur because of both profound neutropenia and the effacement of the intestinal barrier. Anti-neoplastic drug therapy, aplastic anemias, and myeloid tumors may all be associated with severe neutropenias and secondary infections in dogs. Diabetes mellitus in humans and dogs is associated with defects in neutrophil function, and diabetic dogs have a greatly increased incidence of bacterial cystitis (Kirsch, 1998). Hepatic abscesses have also been described in two dogs with diabetes mellitus (Grooters et al., 1994).

Selective suppression of cellular immunity by acquired diseases is very uncommon, because both cellular and humoral immunity are usually effected together. Drugs like Cyclosporine A have much more affect on cellular immunity than on humoral immunity. Corticosteroids can inactivate infections that are kept contained mainly by cellular immunity, e.g. latent ringworm and demodectic mange infections. Splenectomy in dogs can lead to the reactivation of latent *Haemobartonella canis* infection. Chronic corticosteroid treatment frequently leads to bacterial cystitis, especially in female dogs.

Canine Distemper Virus infection can induce a severe combined T- and B-cells immunodeficiency. Dogs with distemper often have intractable secondary bacterial infections of the lungs and intestinal tract and other unusual types of infection such as neosporosis. Indeed, chronic distemper in older dogs in shelters and kennels may manifest as an immunodeficiency rather than a neurologic problem.

Acquired deficiencies in humoral immunity are uncommon. Immunoglobulin secreting myelomas or lymphosarcomas are frequently associated with profound depressions of other immunoglobulin classes. Acquired hypogammaglobulinemia has been described occasionally in dogs, but is relatively common in humans. Affected individuals have a delay in their production of immunoglobulins. They are usually normal until maternal immunity declines, and then suffer from systemic bacterial infections. The defect usually corrects itself with time.

Failure of passive systemic immunity is a serious problem in foals and calves, but less common in puppies. Nevertheless, puppies that fail to nurse and are reared on milk replacements lack passive systemic and local immunity. Such puppies are very susceptible to overwhelming intestinal or blood infection of bacterial origin.

8. Graft immunity

Tissue and organ transplantation is now routine in human medicine and is being applied on a limited basis in veterinary medicine. The greatest hurdle preventing the widespread use of organ transplantation in man and animals is the problem of tissue incompatibility and graft immunity.

The body has complex immune mechanisms that recognize and destroy tissue that is foreign to itself. Animals have developed mechanisms for identifying and destroying living tissue from other species (xenogeneic tissue) for understandable reasons. Complex microorganisms such as parasites, bacteria, fungi, protozoa, and viruses are essentially xenografts trying to establish themselves in the body. Failing to recognize their foreignness could cause death of the animal. When xenogeneic tissue, such as grafts of mouse or cat tissue, are implanted in a dog, they are treated as if they were invading microorganisms.

Why the body has developed mechanisms to recognize and destroy tissue from members of its own species (allogeneic tissue) is not as easy to understand. Individuals of one species do not normally invade the bodies of other members of the same species, so alloimmunity seems unnecessary. It has been theorized that self-recognition is important for preventing autoimmunity and the emergence of mutant cells that could harm the body (e.g., cancers). Graft rejection may also be a necessary side effect of MHC diversity. Diversity in the MHCs is essential for the survival of species when exposed to new infections.

With the exception of pregnancy, where allogeneic tissue is not attacked by the host, allogeneic immunity is in continuous operation and complicates the whole approach to medicine. If it were not for allogeneic immunity, organs could be freely and easily exchanged from one individual to another. Because of allogeneic immunity, surgeons must spend much of their time patching up old parts, bypassing defective parts whenever possible, or replacing faulty parts with new ones made of plastic, metal, or other substances that will not evoke a host response.

The trigger of tissue incompatibility rests in the structure of proteins found on the surfaces of cells. These proteins allow the immune system to discern whether the tissue is its own (autologous or syngeneic), of the same species but from a different individual (homologous or allogeneic), or from organisms of a different species altogether (heterologous or xenogeneic). The greater the difference between the antigens of the tissue and those of the host, the more immunogenic the tissue will be, and the more rapidly it will be recognized and destroyed. Although living xenografts are rarely given, xenogeneic tissues such as pericardium or catgut are widely used in surgery. Such grafts, however, serve only as scaffolds that will be repopulated by host tissue. Their immunogenicity is greatly diminished by chemical treatment.

The allograft reaction is much stronger in the dog than in virtually any other species, including humans. This does not appear to be due to a greater degree of genetic diversity among dogs, because genetic diversity within and between breeds is surprisingly small. Rather, the violence of allograft rejection in dogs seems to be some intrinsic property of allograft immunity in the species.

A great deal is known about the major histocompatibility antigens of dogs, because of the use of dogs in human transplantation research. The antigens on dog cells that define allogeneic differences are found on many cell types. In grafts, alloantigens are rich in passenger host lymphocytes and on graft endothelium. Leukocytes, being particularly rich in histocompatibility antigens and easy to collect from the blood, were the tissue of choice for early researchers. These early researchers used alloantibodies and mixed-lymphocyte reactions to define histocompatibility differences between individuals. Antigens that define histocompatibility differences are coded for by genes within the major histocompatibility complex (MHC) and are called dog leukocyte (DL) antigens. The genes of the MHC are found on a single chromosome. The first group of DL antigens that have been defined are called A, hence the term DLA antigens. Current designations of genes and alleles of the MHC in the dog are increasingly derived from actual DNA sequence.

The DLA genes belong to either MHC class I or MHC class II. Class I genes were initially defined by serology, while class II genes were based on mixed lymphocyte reactivity (Bull et al., 1987; Vriesendorp et al., 1971, 1973; Vriesendorp, 1979). Several sequence-based studies of canine MHC type I genes have been reported (Burnett et al., 1997; Graumann et al., 1998; Wagner et al., 1999).

There are at least four MHC class I genes: DLA-88, DLA-12, DLA-64, and DLA-79 (Wager et al., 1999). DLA-88 has at least 40 alleles, while the other class I loci have fewer than 12 alleles. Within the dog MHC class II, region, clear homologues have been identified for human DRA, DRB, DQA, and DQB (Kennedy et al., 1998a, b; Polvi et al., 1997; Sarmiento et al., 1990, 1992, 1993; Wagner et al., 1996a, b, 1998). There are two genes in the DQ region, DQA1 and DQB1, and one gene in the DR region, DRB1. Using DNA sequence data, over 40 different alleles have been identified in the DLA-DRB1 gene, 18 alleles at the DLA-DQA1 loci, and over 20 alleles in the DLA-DQB1 gene (Kennedy et al., 1998a, b).

In addition to the regions making up the MHC class I and II genes, adjacent regions contain genes that code for proteins which regulate the immune response. These immune response (Ir) genes have been called IrGA, IrGL, and IrGT.

As in humans, the histocompatibility complex in dogs is inherited as blocks; one block (haplotype) from the sire and one from the dam. If two siblings possess the same serologically or DNA sequence defined DLA antigens at one loci, they will also possess the identical haplotype. The greatest similarity between donor and recipient tissues will therefore occur in DLA-identical siblings and the least identify will occur in DLA-different unrelated dogs.

First-set rejection is the immune response that is directed against a graft in a previously unsensitized individual. It usually occurs over a period of 7–14 days when histocompatibility differences are great, or it can take several weeks when histocompatibility differences are weak. It begins as soon as the graft is vascularized in the donor. Donor lymphocytes (present in the graft at time of donation) and the graft endothelium express significant surface concentrations of histocompatibility antigens. Donor or passenger lymphocytes contain the highest level of alloantigens on their surfaces, while normal resting donor endothelium has a much lower amount. Recipient T-lymphocytes surveying the tissues rapidly detect these differences and bind to the surfaces of these cell types. What follows is essentially a delayed type hypersensitivity reaction targeted against donor lymphocytes (passenger lymphocytes) and the graft endothelium. The latter tissue is the essential target of the response, although passenger lymphocytes may play an important primary sensitizing role. Activated lymphocytes specific for the alloantigens of the graft began to bind in low numbers to the graft endothelium and release their cytokines. One effect of the cytokines is to cause the graft endothelium to become activated, which upregulates the surface expression of the alloantigens. This increase in surface alloantigen expression makes the graft endothelium much more vulnerable to attack by immune lymphocytes. As more and more activated T-cells bind to the endothelium, the mass of cytokine release causes eventual death of the endothelium. With the death of the endothelium, thrombosis occurs and the graft dies.

RBC antigens may also be present on graft endothelium and act as targets for rejection. Therefore, donors and recipients are always matched for RBC types prior to grafting. Because there are far fewer RBC antigens than leukocyte antigens, this is not as big a problem as leukocyte matching.

Towards the end of first-set rejection, antibodies against the alloantigens are also produced. These antibodies bind to the endothelium and fix complement and have a destructive effect on the graft endothelium.

Allografts placed into individuals that have been pre-sensitized to leukocyte alloantigens on the graft will be rejected within 24–72 h by largely antibody-mediated destruction of the endothelium, a process known as second set or acute rejection. This is because the blood of the recipient contains high levels of cytotoxic alloantibodies in addition to immune lymphocytes. Pre-sensitization to leukocyte antigens can occur from previous grafting, previous blood transfusions, or through pregnancy. Second-set rejection can also occur when grafting against the RBC types, especially if the recipient has natural cytotoxic antibodies to the RBC type of the donor.

Whole organ allografting is very uncommon in client owned dogs as a routine procedure, but is increasingly being done on an experimental and individual basis. Siblings with the same DLA type (as determined serologically or by DNA sequencing) can be successfully engrafted with a minimum of anti-rejection treatment. However,

mismatched transplants are vigorously rejected, unless intensely treated with certain drugs. The most significant breakthrough in transplantation in dogs may have come with the discovery of leflunomide. Leflunomide, when used in combination with prednisone and cyclosporine A, has allowed long-term maintenance of grafts between unrelated dogs for the first time (Lirtzman et al., 1996).

Pancreatic β-cell transplants have been successfully done in dogs by placing the purified β-cells in small semi-permeable chambers in the abdomen. These chambers allow the entrance and exit of soluble nutrients and hormones, but not of immune cells.

9. Therapy of immune-mediated diseases

Several reviews on the application of 'immunosuppressive therapy' in dogs have been published (Miller, 1997a, b) but are no longer up to date with newer treatment strategies. A simple review of current immunosuppressive therapies is provided by Janeway and Travers (1996).

Treatment of immunologic disorders is directed at two basic areas: (1) the cause of the abnormal immune reaction; or the (2) effect of the abnormal immune reaction. For instance, in the treatment of a food allergy, a hypoallergenic diet would be directed at the cause, while steroid therapy without a change in diet would be directed at the effect (the type I mediated inflammation). Whenever possible, treatment should be directed at the cause of the abnormal immune reaction and not the end result.

Drugs used to treat immunologic diseases are often called 'immunosuppressive'. However, it is not technically correct to say that all such drugs work through suppressing the immune response. In many cases, the ultimate mode of activity is one of immunomodulation or immunoregulation, where perturbed immune responses are modulated back into normal balance. Most of the immunosuppressive drugs that are used were not developed with this activity in mind, e.g., glucocorticoids, cytotoxic agents. gold salts. Rather, they were initially used decades ago for their anti-inflammatory (steroids, gold salts), anti-tumor cell (cyclophosphamide, chlorambucil, azathioprine, vincristine,), or hormonal (danazol, megesterol acetate) activities. However, with the recent explosion of knowledge on the inter-workings of the immune system and various lymphokines and cytokines, drugs with specific effects on the immune system have been developed such as cyclosporine, mycophenolate mofetil, tacrolimus, rapamycin, mizoribine, brequinar and leflunomide (ten Berge and Schellekens, 1997).

Immunosuppressive drug therapy is generally more effective in controlling disease in young animals than in aged animals. This is because immune disorders in younger animals often occur in the face of a relatively intact, or even hyperactive, immune system, while immune disorders in older animals often occurs because of degeneration of the immune system. An intact immune system is more amenable to various modulations than an immune system that is deficient.

Accessory drugs (drugs in addition to steroids) should be withdrawn once the disease is in complete remission for at least one month or more, depending on how long it took to achieve remission. During the same period the glucocorticoid dosage also should have been incrementally decreased as clinical improvement occurred. By the time complete

remission of disease occurs, the glucocorticoids should have already been reduced to maintenance levels. If signs reappear when the animal is receiving glucocorticoids alone, re-establish remission with combination therapy, then maintain remission with the lowest possible dosages of glucocorticoid and cytotoxic drugs. If the disease remains in remission for at least two to three months on steroids alone, the glucocorticoids can be withdrawn. The biggest mistake made is to decrease drug dosages too rapidly and to follow a rigid time schedule for treatment. As long as steady, albeit slow, improvement is seen, vigorous therapy should be continued. Decreased levels or discontinuation of drugs should only follow substantial degrees of improvement. Therapy should never be completely discontinued when there is active disease present, even though it appears slight.

9.1. Glucocorticoid therapy

Almost all treatment regimens have a glucocorticoid as the sole treatment agent, or as the main drug in a combination of agents. This is because glucocorticoids strongly synergize with the anti-inflammatory and/or immunomodulating effects of a number of agents, including cytotoxic drugs, gold salts, and even specific immunosuppressives like cyclosporine. Interestingly, non-steroidal immunosuppressive drugs usually do not work nearly as well as glucocorticoids as sole therapeutic agents. Therefore, immunologic diseases that do not respond even partially to high dose glucocorticoids will probably not respond very well to combination drug therapy. Conversely, if there is partial response to high dose glucocorticoids, there will usually be an enhanced or synergistic response to combination therapy.

Combination therapy is used under three general circumstances:

1. when there is only a partial response to steroid therapy alone;
2. when disease can only be controlled with levels of glucocorticoids that are detrimental to the animal's health; or
3. when it is desirable to achieve disease remission more rapidly;

When several drugs are used in combination, the effective dosage of each drug is often reduced. This reduces toxicity while retaining or even enhancing total therapeutic efficacy. The obvious question is 'when should glucocorticoids be used alone, and when should combination immunosuppressive therapy be initiated?' This decision is based on the familiarity of the veterinarian with the use of these drugs and his/her experience with treating a number of actual cases of the disease in question. Many veterinarians, however, will rely on high dosages of glucocorticoids to control conditions that can be more effectively and safely treated with combination therapy. These same veterinarians are also willing to accept partial control of diseases using steroids alone, even though better control could have been achieved with combination therapy.

Prednisone, prednisolone, and dexamethasone are the most commonly used glucocorticoids in combination immunosuppressive drug therapy. The main differences among these glucocorticoids are in their relative potencies; dexamethosone is around seven times more potent per mg than prednisone or prednisolone. Prednisone is

metabolized to prednisolone in the body, and the latter is the active moiety. Therefore, there might be some advantage to giving prednisolone over prednisone, especially if there are liver problems. In reality, the two drugs can be used interchangeably.

Glucocorticoids exert their immunosuppressive effects in various ways. Glucocorticoids are not highly suppressive to normal primary and secondary antibody responses at therapeutic dosages, although they do seem to markedly depress abnormal autoantibody formation (i.e., Coomb's antibody, antiplatelet antibodies, antinuclear antibodies, and so on). In this regard, they appear to have a favorable regulating effect on events leading to abnormal antibody production. Glucocorticoids cause a pronounced lymphopenia, which limits the number of these cells available for participation in cell-mediated processes. Glucocorticoids may reduce specific antigen-induced blastogenesis of lymphocytes. These drugs produce a monocytosis, but diminish the response of these cells to some inflammatory mediators. Monocyte function in vitro and in vivo is decreased. This effect is manifested systemically by a decrease in the uptake of opsonized and nonopsonized materials by the reticuloendothelial system and locally by a decreased influx of monocytes into inflammatory sites. The reticuloendothelial blockade effect has obvious benefits in the control of red cell and thrombocyte destruction in autoimmune hemolytic anemia and thrombocytopenia.

Glucocorticoids inhibit the production of interferons, and interleukins-1 and -2, and affect neutrophil function. They increase blood neutrophil levels by stimulating their release from the marrow; paradoxically, glucocorticoids inhibit the migration of neutrophils from the blood into the inflammatory site. They also impair neutrophil chemotaxis, adhesiveness, bacterial killing, and lysosomal enzyme secretion in vivo. Glucocorticoids greatly reduce the number of circulating eosinophils.

There are several miscellaneous effects of glucocorticoids that may be of benefit in the treatment of immunologic diseases. Glucocorticoids appear to depress serum complement levels. They also interfere with the passage of immune complexes through basement membranes. Both of these effects have obvious benefits in the treatment of immune complex-mediated disorders.

Corticosteroids should be used at the highest dosage levels during the first 1–2 weeks to control the disease and then decrease the dosage slowly and stepwise at 2–4 week intervals as long as there is a continuous and significant improvement. Improvement should be gauged by measurable parameters such as RBC numbers, platelet numbers, joint fluid cytology, etc., and not just outward signs of health. The initial dosage should not exceed 2.0–4.0 mg/kg PO per day, except in very small dogs and cats, in whom the dosage may be greater. When giving these initial high levels, it is beneficial to administer the drug in divided daily doses.

Once the clinical signs have disappeared, glucocorticoid levels should be decreased over a several weeks to maintenance levels. Maintenance levels of prednisone or prednisolone should be $\approx$1.0 mg/kg PO every other day, or lower if possible. Do not discontinue glucocorticoid therapy for at least one to three months after the disease is in remission; the longer it takes to achieve remission, the longer the animal should be maintained in remission. If drugs are discontinued too soon the disease is more likely to recur and the disease-free interval will often be shorter.

9.2. Cytotoxic drugs

Glucocorticoids are often used in conjunction with any one of a number of different cytotoxic drugs. The term 'cytotoxic drug' refers to the drug's ability to kill cancer cells, for which most of them were initially developed. Cytotoxic drugs also have potent effects on the immune system and are often anti-inflammatory as well. Cytotoxic immunosuppressive drugs used in veterinary medicine belong to three basic groups: alkylating agents; thiopurines; and vinca alkaloids. Folic acid antagonists, such as methotrexate, compose a fourth group. Methotrexate, however, is too toxic to be considered for routine use.

9.2.1. Alkylating agents

Alkylating agents have been found to be among the most potent immunosuppressive drugs available. Alkylating agents work by cross linking the DNA of both dividing and resting cells. They also alkylate nucleic acids; alkylated guanine will pair with thymine instead of cytosine, thus causing a misreading of the genetic code. They produce most of their effects, therefore, by interfering with cell division. Cyclophosphamide (Cytoxan) and chlorambucil (Leukeran) are the most potent immunosuppressive agents in this group. Alkylating agents act against many arms of the immune response. They inhibit normal primary and secondary responses as well as delayed hypersensitivity reactions. They prolong the survival of organ grafts, decrease the production of interferon, diminish antigen trapping in lymph nodes, and inhibit local inflammatory responses. Alkylating agents act on lymphoid cells by killing slowly proliferating antigen reactive cells as well as rapidly proliferating specifically stimulated cells. As such, they inhibit antibody synthesis from 48 h before to a week or more after antigen administration.

Cyclophosphamide is the most popular of the alkylating agents. It is usually given at a dosage based on the body surface area rather than on body weight. This is also true of the other cytotoxic drugs that will be discussed later. Larger dogs require less of the drug per kilogram of body weight than small dogs and cats. Cyclophosphamide is given at a dosage of 1.50 mg/kg PO daily for dogs >25 kg, 2.0 mg/kg PO daily for dogs 5–25 kg, and 2.50 mg/kg PO daily for dogs under 5 kg and cats. Cyclophosphamide is given daily for four consecutive days of each week, followed by three days with no drug; this is referred to as one cycle of therapy. Cyclophosphamide can also be given at the previously mentioned dosage on an alternate-day basis.

Chlorambucil has been much less utilized than cyclophosphamide for the treatment of immunologic diseases. In humans, it has been most effective in the treatment of a number of glomerulonephritides, such as lupus nephritis and idiopathic nephrotic syndrome of children. In these diseases of humans, chlorambucil has been shown to be much more effective than cyclophosphamide and has fewer side effects. The impression in animals has been that it is less effective than cyclophosphamide as an immunosuppressive agent, but that the decrease in efficacy is compensated for by its lesser toxicity. This drug will probably see increasing usage in veterinary medicine for the treatment of immunologic diseases as clinicians learn to use it to treat more conditions. Chlorambucil is given at a dosage of about 1 mg/15 kg (0.07 mg/kg) PO, once daily, for animals in the 15- to 30-kg weight range. Smaller dogs should receive about 0.08 mg/kg and larger dogs about

0.06 mg/kg PO once daily. After remission is achieved, the drug should be administered at the previously mentioned dosage once every other day.

Cyclophosphamide and chlorambucil have several potential side effects at the prescribed dosages. They can be bone marrow suppressive in some animals. For this reason, blood counts should be done every two weeks for the first two months of therapy and monthly or bimonthly thereafter. The white blood cell counts should not be allowed to go lower than 5000/ul. If this occurs, the drug should be briefly discontinued and reinstituted at three-fourths the original dose. A sterile hemorrhagic cystitis is a common side effect of chronic cyclophosphamide therapy. For this reason, cyclophosphamide should not be used for periods longer than three to four months. If cytotoxic drug therapy is required after this period of time, chlorambucil or azathioprine should be substituted for cyclophosphamide. Neither of these drugs induces sterile hemorrhagic cystitis. Cyclophosphamide and chlorambucil retard new hair growth in shaved areas.

9.2.2. Thiopurines

Thiopurines achieve their effect by competing with adenine in the synthesis of nucleic acids. The net result of the substitution of nucleic acids with these 'non-sense' bases is an inoperable nucleic acid strand. Thiopurines, therefore, prevent the proliferation of rapidly dividing cell populations. This differs from the effect of cyclophosphamide, which works against resting and slowly dividing cells as well.

Azathioprine (Imuran) and 6-mercaptopurine (Purinethol) are the most widely used of the thiopurine compounds. Azathioprine is cleaved in the body to two molecules of 6-mercaptopurine, which is the active moiety. These two drugs can, therefore, be used interchangeably. The thiopurines strongly inhibit T-lymphocyte-related functions such as cell-mediated immunity and T-lymphocyte-dependent antibody synthesis. For optimum effect, they must be given during the stage of immune response in which cells are rapidly dividing. The thiopurines have been useful in preventing allograft rejection and have a particularly strong inhibitory effect on the expression of delayed hypersensitivity. They also have a potent inhibitory effect on the non-specific inflammatory response, which may be due in part to inhibition of the influx of mononuclear cells into the site. The thiopurines are potent inhibitors of the primary humoral antibody response. Inhibition of the secondary antibody response requires higher levels of the drugs.

The thiopurines have been used successfully to treat many autoimmune disorders of humans. These drugs have been beneficial in the treatment of autoimmune hemolytic anemia, pure red cell aplasia, Crohn's disease, pemphigus vulgaris, systemic lupus erythematosus, lupoid hepatitis, chronic active liver disease, Goodpasture's syndrome, allergic vasculitis, Wegener's granulomatosis, polyneuritis, and rheumatoid arthritis. Thiopurines have been used in a number of diseases of dogs, such as immune cytopenias, idiopathic polyarthritis, systemic lupus erythematosus, and the bullous dermatitides.

The dosages for azathioprine and 6-mercaptopurine are the same, about 2.0 mg/kg PO once daily. When the disease is under control, alternate-day therapy is used at the dosage previously mentioned. With alternate-day therapy, the glucocorticoids and the thiopurines are given on consecutive days. Used at the recommended dosages, azathioprine and 6-mercaptopurine are safe drugs. They are potentially bone marrow suppressive (Rinkardt and Kruth, 1996) and gastrointestinal toxic at higher levels. As with the use of all

cytotoxic drugs, it is recommended that the white blood cell counts be monitored every 1–2 weeks for the first 6–8 weeks of therapy and not allowed to fall below 5000/ul. If the white cell counts fall below this level, the drug should be discontinued for 1–2 weeks and reinstituted at 1/2 to 3/4 the dosage. Like the alkylating agents, the thiopurines retard hair growth.

9.2.3. Vinca alkaloids

Vinca alkaloids are used for the treatment of certain cancers in animals and humans. Vincristine (Oncovin) is the most widely used drug of this group. Vincristine inhibits the formation of the cytoplasmic microtubular network and prevents cell division by interfering with the formation of the mitotic spindle. Because phagocytosis also involves the cytoplasmic endoskeleton, this is also effected. The vinca alkaloids have been reported to have only 1/10 to 1/100 the immunosuppressive effect of drugs such as cyclophosphamide. Their most potent immunosuppressive effect is at nearly toxic levels. The sole use of vincristine in immunologic disorders is the treatment of autoimmune thrombocytopenia in humans and animals. In addition to its effect on phagocytosis of abnormal platelets, the drug has a curious thrombocytotic property that actually increases the production of platelets.

The dosage of vincristine for dogs is about 0.02 mg/kg IV once a week. If it is going to be effective, a dramatic rise in the platelet count will occur within four to six days after the first injection or several days after the second injection. Therapy is discontinued if no response is seen after the second dose. Vincristine is recommended for use in cases of thrombocytopenia in three situations:

1. in animals with severe blood loss; vincristine often gives a much more rapid response than prednisone or prednisolone alone;
2. in animals with a poor or negligible response to glucocorticoids; or
3. in animals that fail to respond to glucocorticoids and splenectomy.

Vincristine and splenectomy have an apparent additive effect, even if no response occured to splenectomy alone. Most cases of thrombocytopenia of immune origin respond well to glucocorticoids alone and do not require vincristine therapy. Even when vincristine is used, it should be combined with glucocorticoid therapy to facilitate and maintain remission once it is achieved. Side-effects are generally less than other cytotoxic drugs, especially when limited to one or two doses. Side-effects include severe perivascular reactions if injected out of the vein, leukopenia with a nadir 4–10 days after injection, and peripheral neuropathy.

9.2.4. Gold salts (crysotherapy)

Gold is the oldest of all immunosuppressive drugs and was first used by Koch in 1890 for the treatment of tuberculosis. It was later used with some success for treatment of SLE and RA, thought at one time to be forms of tuberculosis.

Gold is available as aurothioglucose (Solganol, Schering) or gold sodium thiomalate (Mycochrysine, Merck). Aurothioglucose has less acute anaphylactic reactions and is the drug of choice. It is available as a suspension containing 50 mg/ml of gold. The

dosage for dogs is 1 mg/kg IM every week for six to eight weeks, then 1 mg/kg once a month.

Gold is excreted from the body in the urine (50–85%) and in the feces. Only 30–50% of the administered dose is excreted each week. The level of gold in the body progressively increases, therefore, with each weekly dose. Gold levels are maintained thereafter by the once monthly injections. Residual gold can still be found years after therapy ceases.

The precise mode of action of gold salts is unknown. The maximum therapeutic effect often takes from six to eight weeks or more to obtain. Gold stabilizes lysosomal enzymes, inhibits certain leukocyte enzyme systems, decreases the migration of phagocytic cells into sites of inflammation, diminishes phagocytic activity, inactivates complement components, inhibits prostaglandin synthesis, inhibits antigen and mitogen induced lymphocyte proliferation, suppresses immunoglobulin synthesis, and stabilizes collagen. It has also been found to decrease immunoglobulin levels, rheumatoid factor levels, and autoantibody levels in pemphigus foliaceus.

Chrysotherapy is used as an adjuvant to glucocorticoid and glucocorticoid–cytotoxic drug therapy. It is seldom effective as the sole immunosuppressive agent. It will, however, reduce the effective therapeutic dose of corticosteroids and in this way reduce cortisone side-effects. Because gold takes many weeks to reach its maximum level of efficacy, it is often combined initially with glucocorticoids and cytotoxic drugs. By the time remission is achieved, the gold is starting to take effect. Once remission is achieved, the cytotoxic drugs are withdrawn and an attempt is made to maintain the remission on glucocorticoids and gold salts alone. If the remission is sustained, the dosage of glucocorticoid is slowly reduced and in some cases withdrawn. If the addition of gold to the therapy fails to provide significant benefit after six weeks of weekly treatments, the drug should be discontinued. If after six weeks a good response is noted, gold treatment can be continued on a monthly basis.

Chrysotherapy has been used to treat the bullous diseases, idiopathic polyarthritis, vasculitis, and rheumatoid arthritis in dogs. In man, the use of gold salts has been counter-indicated for the treatment of SLE. Some dogs with SLE, however, seem to respond favorably to gold therapy.

Less than 5–10% of dogs on chronic chrysotherapy will develop thrombocytopenia, aplastic anemia, toxic nephritis, oral ulceration (stomatitis), and epidermal necrolysis. With the exception of the nephritis, these side-effects will usually clear up when the drug is withdrawn. Recovery can take several weeks or months, however. Additional side-effects in man include dermatitis, proteinuria, GI upsets, neurologic reactions, hepatopathies, and acute anaphylactic reactions.

9.2.5. Cyclosporine A

Cyclosporine is available in injectable (IV) and can be incorporated with olive oil and used as an eye drop. Cyclosporine is readily absorbed into the blood from the conjunctival sac.

The canine dosage is 5 mg/kg IV, q 12 h for several days to establish blood levels and then 10–35 mg/kg PO, q 12 h thereafter. Blood levels achieved after oral administration

vary greatly depending on the individual. Therefore, adjust oral dosage to achieve trough (taken just prior to second date) whole blood levels of about 500 ng/ml.

Cyclosporine is the first of a new generation of immunosuppressive drugs that are relatively specific in their modes of action. Cyclosporine causes T-cells to remain in a resting phase in response to activation signals. It works specifically on T-helper/cytotoxic cells (CD4+ T-cells) and spares T-suppressor cells (CD8+ T-cells). Cyclosporine interacts with cyclophillin to block T-cell signal transduction and activation. It increases the production of IL-1 by macrophages and IL-2 by CD4+ T-cells. Cyclosporine also inhibits B-cell growth factor and inhibits the cidal activity of macrophases in response to γ interferon.

Cyclosporine, used in combination with prednisone, is an effective treatment for autoimmune disorders such as IHA and ITP. Cyclosporine also appears to hold some promise for the treatment of bullous diseases in man and animals. Cyclosporine eye drops have proved effective in one-half or so of the cases of keratitis sicca in dogs. The drug is highly synergistic with glucocorticoids and combination therapy minimizes the toxicity of both drugs and provides maximal therapeutic benefit. Cyclosporine therapy, especially for larger dogs, is currently very expensive. The oral administration of ketaconazole can be used to reduce substantially the oral dose of cyclosporine needed to maintain effective blood levels (Dahlinger et al., 1998); the total cost saving being ca. 25%.

9.2.6. Leflunomide

Leflunomide was developed originally as an anti-inflammatory drug, but was found to be far more active against a wide range of immune disorders in murine models. These models include asthma, SLE, chronic graft versus host disease, autoimmune tubulointerstitial nephritis, anti-basement membrane glomerulonephritis, allergic encephalomyelitis, autoimmune uveitis, and anti-allograft and anti-xenograft rejection (Eber et al., 1998; Waer, 1996; Wennberg et al., 1997; Xu et al., 1997; Yeh et al., 1996, 1997). The drug significantly decreases disease-specific antibody formation. Leflunomide has also shown great promise in the treatment of rheumatoid arthritis in people (Mladenovic et al., 1995; Rozman, 1998), and is undergoing trials for SLE (Strand, 1997).

Leflunomide has an antiproliferative effect on T-cell dependent and independent antibody synthesis (Silva Jaunior and Morris, 1997). The drug has no effect on the release of cytokines, but antagonizes the action of interleukins-3 and -4, and G-CSF, GM-CSF and TNF-α (Cao et al., 1996). It has no effect on IL-1 or IFN-γ. Recently, leflunomide has been shown to inhibit pyrimidine synthesis, and this inhibition can be reversed by free uridine (Fox, 1998; Zielinski et al., 1995). Leflunomide also inhibits cytokine- and growth factor receptor-mediated tyrosine kinase activity (Silva Jaunior and Morris, 1997; Xu et al., 1995).

Leflunomide has many potential uses in veterinary medicine. When used with prednisone and cyclosporine A, it will virtually eliminate allograft rejection responses. It has made it possible to transplant dogs, which are notorious for the violence of their rejection responses, even with prednisone and cyclosporine A treatment (Lirtzman et al., 1996). Leflunomide has also shown great promise in the treatment of immune diseases in dogs (Gregory et al., 1998). One of the metabolites of Leflunomide, trifluromethylaniline

(TFMA), is highly gastroenterotoxic for dogs. Therefore, the dose and dosage regimen must be closely followed. The drug is teratogenic in rats, so long-term use may lead to an increase in certain neoplasms. The dose for dogs is ≈2–6 mg/kg/day per os until a serum trough level of 30 ug/ml is achieved.

9.2.7. Danazol

Danazol is a non-virilizing androgen that has been used for the treatment of refractory immune-mediated hematologic disorders and SLE in man (Cervera et al., 1995). Danazol is thought to work by decreasing the density of Fc receptors on macrophages. Fc receptors bind the Fc portion of platelet bound immunoglobulin and facilitate the macrophage-mediated destruction of the antibody coated platelets. About 20% of the human cases of immune-mediated thrombocytopenia refractory to standard therapy will respond to Danazol within 4–8 weeks, and it has been advocated for use in dogs for this purpose (Miller, 1997). It has also been used to treat a dog with an Evan's-like syndrome (Holloway et al., 1990).

Danazol comes in oral tablets and the dosage is 5–10 mg/kg p.o. daily for at least eight weeks. If no response in eight weeks, discontinue its use. If it proves effective, treat for 4–6 months, and then try to take off drug or reduce dosage. There have been no noticeable side-effects associated with its use in dogs. Although highly touted when first reported, its effectiveness in both humans and dogs has not met expectations.

9.2.8. Intravenous immunoglobulin

Intravenous administration of γ globulin has been of therapeutic benefit in people with IHA and ITP, as well as other immune disorders. In one study, human γ globulin given intravenously has been shown to provide some short-term benefits when given to dogs with IHA, but did not improve long-term survival (Scott-Moncrieff and Reagan, 1997; Scott-Moncrieff et al., 1997). Human immune globulin was also given to 13 of 37 dogs with IHA that had failed to respond to glucocorticoids after seven days; the addition of the globulin appeared beneficial in 10/13 of these animals (Kellerman and Bruyette, 1997). Further studies need to be done using canine rather than human gamma globulin.

9.2.9. Splenectomy

Splenectomy is commonly used for the adult forms of ITP in humans and for some cases of IHA. It should be considered a treatment of last resort in animals, however. Its use should be limited to the following situations: (1) when a satisfactory remission cannot be obtained with single or combination drug therapy alone; (2) when an animal is showing toxicity to the chronic drug therapy needed to maintain remission and either the drugs need to be eliminated altogether or their dosage needs to be decreased. Mixed results have been described for splenectomy in dogs. In one study of IHA, ITP and Evan's syndrome, splenectomy was found to reduce, but not eliminate, the need for immunosuppressive drugs in 8/9 dogs (Feldman et al., 1985). In another study of dogs with ITP, 4/5 dogs with relapsing and hard to medically control disease were able to go off of all drugs after splenectomy (Jans et al., 1990).

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