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Immune-Mediated Diseases of the Blood

W. JEAN DODDS

Division of Laboratories and Research, New York State Department of Health, Albany, New York

I. Introduction

The immune-mediated and true autoimmune diseases of humans and animals comprise a group of poorly understood disorders in which antibodies are produced against tissues of the body, thus violating the

premise of immune self-tolerance (Dacie, 1967; Dacie and Worlledge, 1968; Schalm *et al.*, 1975; Dodds, 1977; Tizard, 1977; Halliwell, 1978). In true autoimmune disease, a rare entity, the host's immune system goes awry and reacts against its own body. The mechanism for this failure to recognize self is unclear, so that the prognosis for survival on a long-term basis is frequently guarded or unfavorable. In the immune-mediated situation, in contrast, the chances for recovery are much better if the causative agent or trigger of the immune reaction can be identified and removed. In common usage, the term autoimmune disease is loosely applied to most types of immunological disorders of the blood and tissues, although this is technically incorrect as most cases are, in fact, immune mediated. For purposes of this article, therefore, the term immune mediated is preferred.

One of the oldest recognized immune-mediated diseases of both humans and dogs is called autoimmune hemolytic anemia (Dacie, 1967; Schalm, 1975). The clinical entity was known long before the immunological basis of the pathogenesis was understood (Bielschowsky *et al.*, 1959; Videbaek, 1962; Fialkow, 1964; Dobbs, 1965; Robbins *et al.*, 1969). In autoimmune hemolytic disease (AIHD) the antibodies formed are directed against the red blood cell and are best demonstrated by the direct Coombs' antiglobulin test (Dacie, 1967; Williams *et al.*, 1972; Schalm, 1975; Schultz, 1976; Dodds, 1977; Tizard, 1977, 1978; Halliwell, 1978; Slappendel, 1979). Diagnosis is based on a positive Coombs' test, the finding of a responsive bone marrow, and a history that rules out other underlying causes of hemolysis, such as transfusion and sepsis, as well as intrinsic red cell abnormalities such as pyruvate kinase deficiency.

In addition to producing antibodies targeted against red cells, immune-mediated diseases frequently elicit antiplatelet antibodies with resultant thrombocytopenia (Oski and Naiman, 1966; Bachand *et al.*, 1967; Williams *et al.*, 1972; Wilkins *et al.*, 1973; Karpatkin, 1980). The most commonly affected animal species are the dog, horse, and cat (Dodds and Wilkins, 1977; Halliwell, 1978; Byars and Greene, 1982). On average, about two-thirds of the chronic, recurrent thrombocytopenias of humans and animals have an immunological basis (Williams *et al.*, 1972; Wilkins *et al.*, 1973; Schalm *et al.*, 1975; Dodds and Wilkins, 1977; Halliwell, 1978; Karpatkin, 1980; Wilkins and Hurvitz, 1981; Dodds, 1982). Antiplatelet antibodies and/or thrombocytopenia may be associated with antierythrocyte antibodies and/or anemia, or can occur in the absence of red blood cell involvement. Generally, about two-thirds of cases with red cell destruction show a concomitant involvement of platelets.

There has been a significant increase in the number of dogs recognized to have immune-mediated diseases (Dodds, 1982; R. J. Wilkins and A. I. Hurvitz, 1981, K. Young, 1981, and C. Pertz, 1981, personal communications). These include a number of specific immunological diseases that can affect tissues or organs as well as the blood. The conditions have been both autoimmune, in which the body's immune system forms antibodies against self-antigens, or immune mediated, in which foreign antigenic materials such as viruses and drugs act as haptens and adhere to or alter the surface or body tissues and cells, forming antibodies against the hapten–cellular complex. Both types of immunological disease produce similar clinical signs in the affected individual, because the resultant antibodies destroy the target organs or cells. The target tissues can be the kidneys as occurs in systemic lupus erythematosus (Lewis *et al.*, 1965, 1973; Osborne *et al.*, 1973; Schwartz, 1975), the joints as with rheumatoid arthritis (Newton *et al.*, 1976; Pedersen *et al.*, 1976), the skin as in pemphigus (Slappendel *et al.*, 1970; Halliwell, 1978), the muscles as in myasthenia gravis and eosinophilic myositis (Tizard, 1977), and the thyroid gland as in Hashimoto's disease (Halliwell, 1978). In some cases, other tissues such as the bone, intestinal tract, brain, or nervous system can be involved in destructive processes, and specific names have been given to each type of disorder. When several tissues are affected, the resulting disease is more serious.

II. Clinical Signs

If the immune-mediated disease is destroying red blood cells, the affected animal can become suddenly or gradually anemic and weak. The gums and eye membranes may be icteric, and the urine may be dark brown or dark red in color. Diagnosis is confirmed by blood tests and especially the direct Coombs' test, which is positive at some time during the course of the disease. It is important to perform this test before the animal is treated with corticosteroids, because false negative results can occur once treatment has been initiated. If the immune-mediated disease is destroying platelets, the animal will usually show a bleeding tendency from the skin and mucosal surfaces. Typical signs are small pinpoint bruises (petechiae) in the skin, gums, and eye membranes, nosebleeds, large patchy bruises (ecchymoses) in the skin, and bleeding from the gastrointestinal tract (melena) or into the urine (hematuria). The platelet count is usually less than 150,000 per mm^3, and prognosis depends on the severity of the platelet reduction. Very

low counts (less than 30,000 per mm^3) are quite dangerous, because internal bleeding can be fatal. Curiously, a number of severely thrombocytopenic patients do not show clinical signs of a bleeding tendency unless provoked by stress, trauma, or surgery, despite platelet counts of less than 50,000 (Karpatkin, 1980; Dodds, 1982). Why some individuals fail to bleed whereas others with similar histories and laboratory findings have recurrent problems is not understood. Perhaps the function of those few circulating platelets is enhanced in the former cases, which affords a measure of protection. Diagnosis is confirmed by laboratory tests to detect the presence of antiplatelet antibody in the blood. Again, this test should be done before corticosteroid therapy is given.

III. Diagnosis

A. History

The typical history of affected animals will depend on two factors: whether the onset is gradual or sudden, and whether or not both red cells and platelets are involved. The classical acute case of AIHD is presented with a sudden collapse of a previously healthy animal. The gums are usually blanched and frequently are icteric. When platelets are concomitantly depleted, there may be petechiae spread over the ventral abdomen, gums, and sclera. In isolated cases of thrombocytopenia, the disease is less dramatic. The owner may notice small bruises when grooming the dog, or the veterinarian may observe such bruises as the animal is being examined for a routine checkup or vaccination procedure. Chronic cases of AIHD progress slowly to a nonspecific general weakness, lethargy, and inappetence. Episodes of overt illness are usually preceded by environmental or physiological stresses such as extremes of hot or cold weather, hormonal changes (estrus, pseudocyesis), and other disease processes (especially viral infections). The affected animal is frequently a young to middle-aged adult female with a previous history of reproductive irregularities including heat cycles of varying lengths and intervals of anestrus, silent heats, prolonged estrual bleeding, and pseudopregnancy. The owner may also mention that other family members have had similar problems or a series of undiagnosed chronic or acute and fatal illnesses. Some cases have a family history of chronic allergies, which includes various types of seasonal, chronic dermatitis. The history of other illnesses among relatives may reflect "tumors" of the liver and spleen as well as anemia. Questioning the veterinarian involved frequently reveals that this was nonspecific hepatosplenomegaly of unknown cause. Typically there has been an incomplete workup of the case from a clinical pathological or histopathological standpoint.

A second form of immune-mediated anemia is associated with the presence of cold erythrocyte agglutinins. These are more rare than the usual warm-reactive antibodies that produce the classical AIHD syndrome. Cold antibodies are most active below 20°C and produce microcirculatory failure at the extremities rather than hemolytic anemia. Thus the nose, feet, tail, and tips of the ears are affected with dry gangrene-like lesions caused by intravascular erythrocyte agglutination.

In addition to the nonspecific signs of regenerative anemia, affected animals will have hemoglobinuria and may have hemoglobinemia if intravascular hemolysis is sufficient to exceed clearance of erythrocyte breakdown products by the reticuloendothelial system. There may also be anorexia, pyrexia, polydipsia, peripheral lymphadenopathy, and hepatosplenomegaly in chronic cases. Most animals respond well if they are treated aggressively after the initial onset of signs. In some cases the course of the disease is unpredictable, and in others there is a spontaneous remission.

If the dog with AIHD also has systemic lupus erythematosus (SLE), it will show a variety of other clinical signs at some point (Lewis *et al.*, 1965, 1973). About two-thirds of the cases have concomitant thrombocytopenia, and there is progressive renal failure from immune-complex glomerulonephritis. Additional signs include polyarthritis, polymyositis, skin lesions that blanket the muzzle, pleurisy, and pericarditis. Diagnosis is confirmed by clinicopathological tests.

B. Clinicopathological Tests

1. Red Cell Tests

The diagnosis of immune-mediated blood diseases is confirmed by clinicopathological testing (Schultz, 1976). When the red cells are involved, the resulting disease can be classified according to the type of erythrocyte antibody produced (Dodds, 1977). This classification has been described in detail by Halliwell (1978). Basically the antibodies are divided into the warm- and cold-acting types, the former being much more common. A classification scheme is shown in Table I.

Warm-reacting antibodies give a positive Coombs' antiglobulin test at 37°C, whereas cold-reacting antibodies are Coombs' positive at 4°C

TABLE I

CLASSIFICATION OF IMMUNE-MEDIATED ANTIERYTHROCYTE AGGLUTININS[a]

Type	Characteristics
Warm Antibodies	
Spontaneous agglutinins	Visible in freshly collected blood; probably is occurring *in vivo;* differentiated from rouleaux by persistence upon dilution with isotonic saline; Coombs' test unnecessary; prognosis guarded to poor
In vivo hemolysins	Massive red cell destruction with hemoglobinuria and hemoglobinemia; sudden onset of serious illness with icterus is common; Coombs' test needed to confirm
Incomplete antibody type	Most common form; Coombs' test important; intravascular hemolysis uncommon; splenomegaly common; hemoglobinuria but not hemoglobinemia present; chronic course with gradual onset
Cold Antibodies	
Cold agglutinins	Optimum effect below 20°C; cold weather-induced anemia and hemoglobulinuria; dry gangrene of extremities common; intravascular hemolysis uncommon; Coombs' test negative at 37°C but strongly positive at 4°C; can occur in warm weather
Nonagglutinating cold antibodies	Coombs' test positive at 4°C only; icterus and hemoglobinuria common especially in cold weather; rare

[a]After Halliwell (1978).

and negative at 37°C. In fact, warming of cold-agglutinated blood will usually reverse the process. The preferred form of the Coombs' assay is the direct test, which uses the patient's washed red cells tested against species-specific antiglobulin serum (Dodds, 1977; Tizard, 1977; Halliwell, 1978; Slappendel, 1979). The indirect form of the test, which uses the patient's serum and normal washed red cells, precludes diagnosis if the antibody titer is too low to elicit a positive reaction. Because these antibodies are most destructive at the cell surface, the direct test is more meaningful and reliable.

The red cell agglutinins can be directed at IgG (15–50% incidence), at complement components, especially C3 and/or C4 (30–50% incidence), at a combination of these two (25% incidence), and at other

antibody types such as IgM or IgA, which are rare (Dodds, 1977). In a recent analysis of 371 anemic dogs (Slappendel, 1979), 134 cases or 36% had positive direct Coombs' tests, and of these 11% had IgG antibodies, 31% had IgG + complement antibodies, 55% had complement antibodies, and about 2% had IgM + complement antibodies. In two Coombs'-positive dogs the type of reaction was unclear, and occasionally IgM and/or IgA reactions occurred along with strong IgG and complement antibodies. Eighty-four of the Coombs'-positive dogs had one or more symptoms of hemolysis including hemoglobinemia, indirect hyperbilirubinemia, increased red cell osmotic fragility, and increased fecal excretion of urobilinogen. Most dogs with the IgG + complement-type agglutinins had severe hemolysis, and primary or secondary diseases were present in only half of these. Thus overt anemia was usually associated with dogs having either the IgG or IgG + complement antibodies. Conversely, those cases with only complement antibodies had minimal or no evidence of hemolysis. In nearly all cases there was an associated primary disease such as infection (especially viral), or inflammatory or neoplastic (especially myelo- and lymphoproliferative) diseases. The indirect Coombs' test was also uniformly negative in cases of complement antibodies.

2. *Platelet Tests*

If platelets are the target cells of immune-mediated destruction, one or more specific tests can be performed to determine the presence, type, and amount of antiplatelet activity in the blood.

The first test used routinely to detect antiplatelet activity was the platelet factor 3 (PF3) release test of Karpatkin and colleagues (Karpatkin, 1980). The assay was adapted for dogs (Wilkins *et al.*, 1973; Joshi and Jain, 1976; Jain and Kono, 1980) and other species (Dodds and Wilkins, 1977; Byars and Greene, 1982) by using species-specific platelet-rich plasma and control globulin fractions and substituting plasma for serum as the initial specimen. The latter change was made to reduce the possibility of obtaining false positive results from traces of thrombin in serum being transferred to the extracted globulin fraction. Being a potent promoter of the platelet release reaction, thrombin would cause nonspecific shortening of the clotting time end point in the test (Dodds and Wilkins, 1977). The collective experience of the author with over 200 cases of recurrent thrombocytopenia indicates that this test is the most practical and reliable overall for clinical use in animals (Dodds and Wilkins, 1977). Because 65–70% of recurrent cases of thrombocytopenia have an immunological basis (Karpatkin *et al.*, 1972; Wilkins *et al.*, 1973: Dodds and Wilkins, 1977: Karpatkin, 1980),

a negative test result does not preclude this diagnosis. Serial monitoring may be required to detect the presence of antibody, and/or the circulating antiplatelet titer may be below the detection limits of the test. False positive test results may also be obtained in cases where a secondary tissue-inflammatory or stress-responsive disease is present (e.g., disseminated intravascular coagulation, acute sepsis) or when the test globulin fraction is contaminated with thrombin or endotoxin, which also induces platelet membrane injury and PF3 release (Karpatkin *et al.*, 1972).

Several veterinary clinical pathology laboratories do use assays based on this test (Joshi and Jain, 1976; Jain and Kono, 1980; Wilkins and Hurvitz, 1981), although difficulties in standardizing the test have been encountered (Halliwell, 1978; Jain and Kono, 1980). The most commonly observed problem is an overly shortened clotting time endpoint for the control globulin fractions (i.e., less than 60 sec). This usually indicates that the normal platelet-rich plasma substrate has been activated during collection and/or preparation, thus causing premature release of PF3 and reduction in the amount of PF3 available for release from subsequent exposure to an immune globulin fraction. The ideal clotting time end point of the control specimen should be around 90 sec for dog samples and over 150 sec for equines. Another cause of foreshortening in the control specimens occurs if the globulin fraction used has been prepared by prior pooling of plasma from several healthy animals (Dodds and Wilkins, 1977). Apparently normal globulin extracts from different individuals can be sufficiently diverse to cause nonspecific interaction, inducing PF3 release. The problem is avoided by preparing a series of normal globulin fractions from several healthy dogs and testing each batch separately.

A variety of other tests have been developed in recent years for the identification of immune-mediated thrombocytopenia in humans (Cines and Schreiber, 1979; Hymes *et al.*, 1979; Karpatkin, 1980; Sugiura *et al.*, 1980; Morse *et al.*, 1981, 1982; Myers *et al.*, 1981) and animals (Shebani and Jain, 1983). Some of these, such as the platelet migration inhibition test (Duquesnoy, 1975), the antibody-dependent cellular toxicity test (Gengozian and Rice, 1982), and the lymphocyte transformation test (Wybran and Fudenberg, 1972), measure cellular involvement with antiplatelet activity; others are more specific and quantitate the amount and type of immune globulin bound to the platelet surface (Cines and Schreiber, 1979: Hymes *et al.*, 1979; Sugiura *et al.*, 1980; Morse *et al.*, 1981, 1982; Myers *et al.*, 1981). The latter tests are more sensitive than those based on measuring plasma or serum antibody levels. Some are simple and rapid (Sugiura *et al.*, 1980; Morse *et al.*, 1981, 1982), and results directly correlate with the degree of platelet destruction and severity of clinical disease, whereas plasma levels of antibody do not (Karpatkin, 1980). Unfortunately, such methods are not easily adapted for use in animals because highly purified radio or fluorescent-labeled, species-specific immunoglobulins or the active fragments of immunoglobulins are required. Development of these reagents would be most useful for research purposes but would be impractical for routine use because the assumption of an immune basis can safely be made for the majority of clinical cases of recurrent, severe thrombocytopenia.

Several simplified screening tests for circulating platelet antibodies are also available (Hirschman *et al.*, 1974) and have been used in dogs (Jain and Kono, 1980). Although promising, whether these are not only easier but also equally or more reliable than the established PF3-release test methods remains to be proven.

The antibodies present in immune thrombocytopenia are usually of the IgG type (75%); the remainder are usually IgM or IgA antibodies (Dodds and Wilkins, 1977; Karpatkin, 1980). The type of immunoglobulin involved can be identified by immunoprecipitation with specific immunoglobulins, in which case a positive PF3 test becomes negative upon removal of the specific immunoprecipitate (Karpatkin *et al.*, 1972; Wilkins *et al.*, 1973). This modification of the basic test can be used to confirm positive test results and avoid false positive diagnosis from nonspecific PF3 release.

In addition to specific tests for antiplatelet activity, other aids in establishing the diagnosis include serial monitoring of the platelet count, coagulation assays to rule out thrombocytopenias caused by the consumption coagulopathy phase of intravascular coagulation, bone marrow examination, and other immunological tests (e.g., Coombs' test, LE preparation, antinuclear antibody test) (Halliwell, 1978; Karpatkin, 1980). Generally, the lower the platelet count, the more severe the clinical entity (Karpatkin, 1980). Also, patients with platelet counts below 10,000 to 30,000 per mm^3 are usually destroying platelets preferentially in the liver rather than in the spleen (Dodds and Wilkins, 1977; Karpatkin, 1980; Pearson, 1980), although both organs frequently are involved. Bone marrow evaluations are usually nondiagnostic, because peripheral thrombocytopenia can result from reduced production and/or excessive destruction, utilization, or sequestration (Karpatkin *et al.*, 1972: Wilkins *et al.*, 1973). Thus, normal, reduced, or enhanced megakaryocytic activity may be present in marrow aspirates.

Because about two-thirds of cases of immune-mediated hemolytic

disease also have thrombocytopenia at some point, it follows that patients with immune thrombocytopenia may also have positive direct Coombs' tests. Similarly, patients with SLE frequently show both antierythrocyte and antiplatelet antibodies (Karpatkin, 1980). Thus, tests for these other immunological disorders should also be performed on thrombocytopenic individuals.

3. Tests for SLE

In addition to the tests just described for antierythrocyte and antiplatelet antibodies, patients suspected of having SLE should be tested for the presence of LE cells and antinuclear antibodies (ANA). It may be necessary to monitor the patient on a serial basis to demonstrate the LE-cell phenomenon or ANA. The former test is based on the principle that *in vitro* incubation of antinuclear activity present in the patient's blood coats the nuclear material released from fragmenting cells, opsonizing it for phagocytosis by polymorphonuclear leukocytes. Thus the LE cell is a polymorph that has ingested coated nuclear material. Similar phagocytosis may occur *in vivo*, and about 75% of patients with SLE will have a positive LE test at some stage of their disease.

Improved specificity and sensitivity for the diagnosis of SLE is possible with quantitative ANA tests. Immunofluorescent and radioimmunoassay procedures are the most widely used and are quite specific. These detect the presence of circulating antibodies against native, double-stranded DNA. Antibodies against leukocytes and rheumatoid factor may also be present in SLE cases.

The criteria for establishing the diagnosis of SLE in dogs have been reviewed byHalliwell (1978) and Tizard (1978). These include one or more of the following: Coombs'-positive hemolytic anemia, thrombocytopenia (usually immune mediated), progressive renal failure with proteinuria, slowly progressive polyarthritis, skin lesions typical of SLE and usually on the muzzle, and the presence of LE cells, ANA, polyclonal gammopathy, or rheumatoid factor. Although Halliwell (1978) stresses that diagnosis of SLE is untenable without positive serological evidence of ANA titer greater than 1:100 or strongly positive LE preparation, there have been an increasing number of cases of so-called ANA-negative SLE, in which the clinical signs, other immunological tests, and response to treatment are typical of SLE (Dodds, 1982; R. J. Wilkins and A. I. Hurvitz, 1981, personal communication). Although the question of diagnosis remains to be resolved here, for all practical purposes these can be managed and treated similarly to proven cases of SLE.

C. Serial Monitoring

One mechanism for managing patients with immune-mediated diseases on a long-term basis is to monitor serially their clinical and clinicopathological status. Depending on the severity and nature of the illness, serial testing can be performed on a monthly or bimonthly basis, or it can be scheduled to coincide with each estrual and interestrual period for affected bitches. From the author's experience with several dog families apparently predisposed to immune-mediated anemia and/or thrombocytopenia, laboratory monitoring on a regular basis has been beneficial not only to identify animals newly converted from a negative to positive status, but also to initiate and adjust treatment regimens to optimize control of the disease process (Dodds, 1982).

IV. Findings

A. Family and Breed Histories

As mentioned earlier, we and other groups have experienced a large increase in the number of referrals of canine patients with immune-mediated diseases of the blood. The trend became noticeable in 1979, and the magnitude of the increase can best be appreciated if one considers that our laboratory studied over 200 animals referred for these diseases during an 18-month period between 1980 and 1982. In the past the average had been about one case per week; this increased to two to four cases per week, with the preponderance in the summer months. An apparent breed predilection has also been observed in our laboratory (Table II). The most frequently affected breed is the Old English sheepdog (57 cases), and the clinical course of the disease appears to be more severe and less responsive to treatment than expected.

In addition to certain breed predilections (discussed in detail in Section V,C), specific families of dogs also seem to have an increased tendency to develop these immune-mediated conditions. The following description summarizes our investigations of two purebred dog families with an abnormally high incidence of immune-mediated anemia and thrombocytopenia. Similar data (not shown) have been collected for families of American cocker spaniels and long-haired dachshunds, and are being assembled for the Old English sheepdog.

TABLE II

DOG BREEDS APPARENTLY PREDISPOSED TO IMMUNE-MEDIATED BLOOD DISEASES

American cocker spaniels
German shepherds
Irish setters
Miniature and standard dachshunds
Mininture and toy poodles
Old English sheepdogs
Shetland sheepdogs
Scottish terriers
Vizslas

1. Vizsla Family

A four-generation pedigree of this family of Hungarian vizslas is shown in Fig. 1. The proband, a 5-year-old intact female, was presented to a local veterinarian approximately 2 weeks prepartum with anorexia of 3 days duration. One week later she had a packed-cell volume (PCV) of 18%, a platelet count of 156,000 per mm^3, and a "nonregenerative" anemia. A Coombs' test performed at this time was negative. She whelped 1 week later. Three weeks postpartum a diagnosis of pyometra was made, and the dog was spayed. One week later her PCV dropped to 11%, and a strong reticulocyte response was seen. The Coombs' test was strongly positive against both IgG and complement antibodies. The following week the patient remained Coombs' positive, and she died shortly thereafter. Postmortem histopathology revealed marked bone marrow hyperplasia, primarily of the granulocytic series, and hemosiderosis. The spleen was enlarged and found to be the site of active erythropoiesis and myelopoiesis. Megakaryocytes were present in the liver, as were macrophages filled with hemosiderin and red blood cells. Results of uterine histopathology included cystic endometrial hyperplasia and endometritis.

Six offspring and three siblings of the affected bitch as well as their progeny have been followed serially for evidence of hemolytic disease (Fig. 1). Of interest is the fact that the proband's 2 sisters and all 5 daughters had a history of reproductive abnormalities (irregular estrual intervals, pseudocyesis, infertility), and that 4 daughters and 1 sister were also Coombs' positive. Of 5 males tested in the family, two were Coombs' positive. Thus 9 of 12 tested females and 2 of 5 tested males had positive red cell antiglobulin tests on one or more occasions. Six family members (3 of each sex), including the proband, were also

thrombocytopenic and had positive PF3-release tests for antiplatelet activity (in Fig. 1, dogs, II,2,6, and 9 and dogs III,4,11,14, and 16). One dog, a male, had mild thrombocytopenia but was PF3 negative (dog III,17).

When the proband's daughter (dog III,4) became Coombs' positive and remained so for a total of three serial tests (two in estrus and one between heats), and developed clinical signs during estrus like those of her dam, she was spayed. A pyosanguinous endometritis was found on histopathological examination of the extirpated tissues. Coombs' tests were then performed at regular intervals, and she became Coombs' negative 8 months after surgery and remained so as of 5 years later. The littermate of this bitch (dog III,3) and the proband's sister (dog II,9) were also considered at risk to develop clinical signs referable to immune-mediated hemolytic disease and/or thrombocytopenia, because they became consistently Coombs' positive. Following ovariohysterectomy, both dogs reverted to a negative antiglobulin status and remained so for 2 and 3 years, respectively, as of this writing. In both

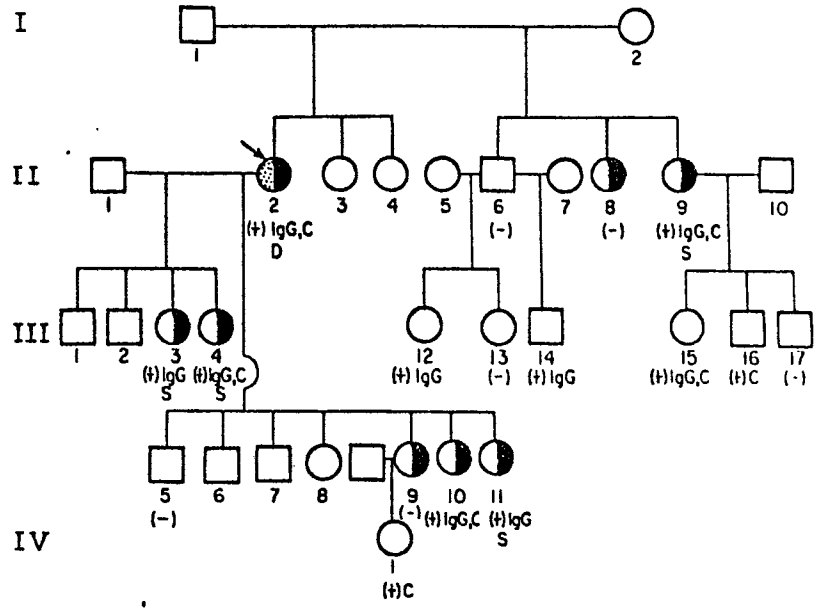

FIG. 1. Pedigree and relevant clinicopathological findings in vizsla family with immune-mediated anemia. The proband is indicated by the arrow. (+), Coombs' positive, IgG or C; (−), Coombs' negative: D, deceased; S, spayed; ◑, reproductive problems; ◑, immune-mediated anemia.

cases, the excised uterine tissues were engorged with uterine fluid. Other females in the family have experienced reproductive problems that include persistent vaginal discharge, irregular estrus cycles and behavior, and positive Coombs' tests. The breeder was advised not to breed females judged to be at risk for immune-mediated disease and to spay them. The immunological status of family members is currently monitored on a continuing basis. Whether spaying those bitches that became Coombs' positive aborted an impending disease problem remains to be proven, but at least none has developed clinical signs and all are Coombs' negative at this writing.

2. *Scottish Terrier Family*

The immunological status of this family of Scottish terriers was followed for several generations (Fig. 2).

The genetic background of this family was originally examined in relation to routine hematological screening procedures for the von Willebrand's disease gene (Dodds, 1980). During this period of testing the breeder was informed that an 8-month-old female puppy from a litter she had bred was acutely ill with a "blood-related" disease. The clinical history and postmortem histopathology suggested an episode of acute hemolytic anemia. A second closely related 11-month-old male

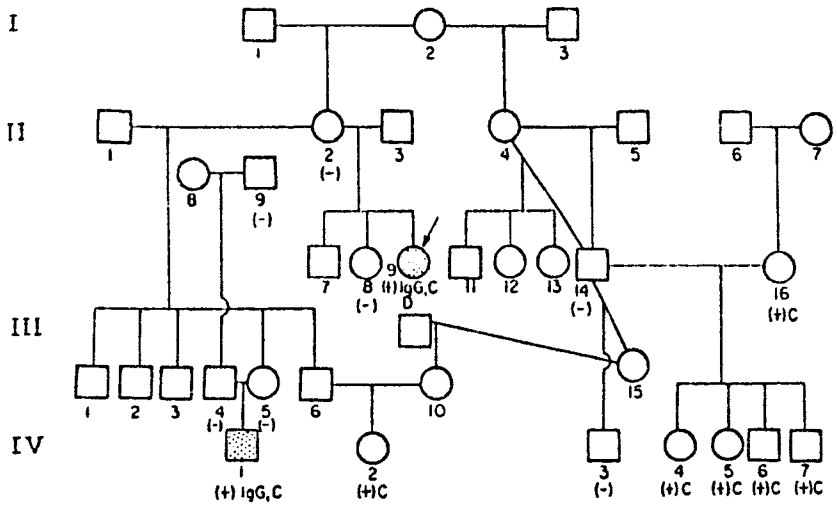

FIG. 2. Pedigree and relevant clinicopathological findings in Scottish terrier family with immune-mediated anemia. The proband is indicated by the arrow. ⊕ or ▨, Immune-mediated anemia; for other symbols, see Fig. 1.

puppy was presented to a veterinary hospital with a history of anorexia and lethargy. On initial examination the dog had a temperature of 104.4°F, pale mucous membranes, a bilirubinuria of 2+, and a PCV of 23%. Blood was submitted for analysis, and the dog was placed on antibiotics. A Coombs' test was not performed. Three days later the PCV had fallen to 8%. The dog received a transfusion of whole blood and underwent exploratory surgery the following day. Twenty-four hours later, with a PCV of 10% and evidence of jaundice, the dog died. Tissues submitted for histopathology revealed hepatitis with focal necrosis, splenitis, hemosiderosis, and extramedullary hematopoiesis.

Coombs' testing was initiated on dogs in the breeder's possession that were related to the two puppies that died. Several Coombs'-positive members were discovered (Fig. 2). Most of the antierythrocyte antibodies were directed against complement components. Eight of these animals were also thrombocytopenic, and five of them were positive for antiplatelet activity by the PF3-release test (dogs III,5,9, and 16 and dogs IV,1 and 2).

Examination of the pedigrees of this breeder's stock revealed a close relationship between the two clinically affected animals; the mother (dog II,2) of the proband (dog III,9) was also the grandmother of affected dog IV,1. Also, the brother (dog III,6) of this male's dam (dog III,5) sired a Coombs'-positive bitch puppy (dog IV,2). The sire of the litter in which all four puppies were Coombs' positive (dog III,14) had the same foundation grandmother (dog I,2) as the proband. Dog III,14 also had been bred back to his mother (dog II,4), but we have not located the three progeny for blood testing.

Since the first cases in Scottish terriers were brought to our attention, several other dogs of this breed with confirmed or presumed immune-mediated hematological disease have been discovered: an 8-year-old male that was euthanized after suffering a severe and unresponsive hemolytic crisis immediately following a second vaccination with modified live parvovirus vaccine of feline origin, a 4-year-old male with marked hepatomegaly that survived a hemolytic episode 2 weeks after receiving a modified live feline parvovirus vaccine, a 2-year-old female with PF3-positive immune thrombocytopenia, and two 6-week-old littermates that succumbed to acute hemolytic disease 9 days following vaccination with a combination of modified live distemper–hepatitis–leptospirosis–parainfluenza and feline parvovirus vaccines. Autopsies revealed distemper virus inclusions in the central nervous system. Blood samples from these puppies and their two surviving littermates were strongly Coombs' positive, with antibodies directed against IgG and complement. Parvovirus titers on the survivors

were subsequently found to be in excess of 1:2000, indicating a recent viral exposure.

The pedigrees of these cases, except for that of the PF3-positive bitch, which was unavailable, were evaluated to search for common ancestry. The two littermate puppies that died after vaccination were not related to any of the others for at least 10 generations. The 4-year-old male with hepatomegaly and the older male were distantly related to the family shown in Fig. 2 through males I,3 and II,5.

B. Case Histories

Table III gives a breakdown of the 223 animals tested for immune-mediated hematological diseases by our laboratory in the 18 months between 1980 and mid-1982. Of these, 57 (26%) involved Old English sheepdogs, 35 (16%) were long-haired dachshunds, 26 (12%) were Scottish terriers, 17 (8%) were vizslas, 15 (7%) were American cocker spaniels, and the remainder were other purebreds (8%) and mixed breeds (23%). Over 70% of these animals were females, which confirms the expected preponderance of females over males with respect to immune-mediated diseases (Dacie, 1967; Williams *et al.*, 1972; Schalm, 1975; Tizard, 1977; Halliwell, 1978). The ages of affected dogs varied from 6 weeks to 15 years, although most animals in this series were of middle age, as has been found previously (Halliwell, 1978).

Of the 214 dogs tested by the direct Coombs' test, 154 (72%) were positive. Of these, the majority (51%) had both IgG and complement attached to their red cells, whereas complement-mediated antibodies were the next most common (38%), with IgG antibodies being least frequent (11%). About one-half (109) of the total number of dogs tested were also thrombocytopenic, and of these, 68 (62%) were positive for antiplatelet activity by the PF3-release test (Wilkins *et al.*, 1973). A striking finding was observed in the Old English sheepdog breed; 39 of the 57 animals tested were thrombocytopenic, and 30 (77%) of these had circulating antiplatelet activity. Many of these affected animals were referred for severe thrombocytopenia without red cell involvement, although some cases also had strongly positive Coombs' tests and were seriously ill.

V. Predisposing Factors

Factors known to predispose to immune-mediated hematological diseases are listed in Table IV.

TABLE III

Animals Tested for Immune-Mediated Blood Diseases, 1980–1982

Breed	Number of animals	Sex (M/F)	Age range	Direct Coombs' test				PF3-Release test	
				(+IgG)	(+C)	(+IgG and C)	(−)	(+)	(−)
Old English sheepdog	57 (9 have same sire)	12/45	5 months to 12 years	5	7	24	18	30	9
Long-haired dachshund	35 (all related)	19/16	12 weeks to 5 years	0	18	5	14	3	7
Scottish terrier	26 (15 related)	7/19	6 weeks to 8 years	0	11	7	9	4	6
Vizsla	21 (all related)	5/16	8 months to 7 years	3	6	6	10	6	1
American cocker spaniel	15 (3 have same dam)	2/13	2–6 years	0	0	8	0	7	0
Other breeds	18	3/15	8 weeks to 14 years	2	4	12	0	3	0
Mixed breeds	51	12/39	5 months to 15 years	6	13	17	9	15	16
Totals	223	60/163	6 weeks to 15 years	16	59	79	60	68	41

TABLE IV

FACTORS THAT PREDISPOSE TO IMMUNE-MEDIATED BLOOD DISEASES

Sex (females 2:1 over males)
Genetic or familial factors
Virus disease and possibly frequent use of modified live virus vaccines
Hormonal influences (pregnancy, abnormal estrous cycles, pyometra, and pseudocyesis)
Drug reactions
Stress (environmental, emotional, or physiological)
Underlying diseases (lymphoreticular malignancies and other autoimmune disorders)

A. INCREASED FREQUENCY

The two- to threefold increase in our caseload between 1979 and mid-1982 was mentioned earlier and documented for the period after 1980 in Fig. 2. By comparison, Halliwell (1978) reported only 21 cases of immune-mediated hemolytic anemia and 15 cases of immune thrombocytopenia referred to the University of Pennsylvania School of Veterinary Medicine for the 6-year period from 1970 to 1976. Of these, there were 3 cockers and 5 poodles with red cell involvement, and 1 Old English sheepdog, 2 cockers, and 4 poodles with immune platelet destruction.

Nearly all the immune-mediated diseases studied have affected the blood at one time or another during the course of the disease. Other commonly associated problems were hepatosplenomegaly, hepatitis, splenomegaly, glomerulonephritis, and dermatitis. Occasionally (six cases) there was a painless and gradual sloughing of the toenails of all four feet. In each of these latter instances, the animal had received a modified live parvovirus vaccination of either feline or canine origin from 5 to 17 days beforehand.

An important question yet to be resolved is whether the observed increase in the number of referrals reflects a true increase in the frequency of these diseases and/or an increased awareness among dog owners, breeders, and veterinarians, who are recognizing and diagnosing such disorders more readily. Certainly, access to the laboratory tests required for diagnosis of immunological disorders in animals has improved in recent years, and this has facilitated accurate diagnosis and early treatment. It is our opinion, however, that the current influx of cases exceeds the number that can be explained on the basis of increased awareness alone.

B. SEX PREDISPOSITION

The predisposition of females to immune-mediated disease has been well established (Dacie, 1967; Williams *et al.*, 1972; Schalm, 1975; Dodds, 1977; Tizard, 1977; Halliwell, 1978; Karpatkin, 1980). This applies not only to SLE and immune-mediated anemias (Williams *et al.*, 1972) but also to thrombocytopenias of immunological causes (Karpatkin, 1980). The present canine data (Table III) are in agreement, because 163 of the animals tested were females whereas only 60 were males. Curiously, affected males—especially of the Old English sheepdog, dachshund, and Scottish terrier breeds—were very severely affected, and many were nonresponsive and succumbed despite vigorous treatment. In accordance with our previous studies (Wilkins *et al.*, 1973; Dodds, 1977), females were more often affected than males by a 2:1 or 3:1 ratio, whether intact or spayed.

C. BREED PREDISPOSITION

Breeds recognized by our laboratory and others to be predisposed to immune-mediated hematological diseases are listed in Table II (Wilkins *et al.*, 1973; Dodds, 1977; Halliwell, 1978; M. Estrin, 1982, personal communication). Data from specific dog families within the more commonly affected breeds have been collected for vizslas (Fig. 1), Scottish terriers (Fig. 2), American cocker spaniels, and long-haired dachshunds, and are presently being compiled for the Old English sheepdog. Whether certain bloodlines within these breeds will be found to have a significantly increased prevalence of immune disorders remains to be proven by statistical evaluation from pedigree analysis of the relationship between affected individuals. One way to accomplish this would be to compare the coefficients of inbreeding and commonality of ancestry of affected individuals to age- and geographically matched healthy animals of the same breed.

D. GENETIC INFLUENCES

Familial tendencies are known to exist for several immune-mediated disorders of humans and animals including rheumatoid arthritis, Hashimoto's thyroiditis, SLE, AIHD, immune-mediated thrombocytopenia, and agamma-globulinemia (Bielschowsky *et al.*, 1959; Videbaek, 1962; Fialkow, 1964; Dobbs, 1965, Lewis *et al.*, 1965; Dacie, 1967; Robbins *et al.*, 1969; Schwartz, 1975; Dodds, 1977; Utroska, 1980). Hemolytic anemia can be associated with all types of immune-mediated disease and may be the first or only presenting clinical sign

in SLE. In NZB/NZW hybrid mice, the AIHD can be directly transmitted from one generation to the next with clinical signs expressed by 6 months of age (Bielschowsky *et al.*, 1959; Mellors, 1969). Affected females are highly susceptible to a nephritis similar to that found in patients with SLE.

In the human medical literature, several families are described with a history of AIHD (Videbaek, 1962; Fialkow, 1964; Dobbs, 1965: Dacie, 1967) or immune-mediated thrombocytopenia (Karpatkin, 1980). In one such family, the proband suffered from AIHD and had a strongly positive Coombs' test. The family history included members with increased levels of immunoglobulins, rheumatoid factor, ANA, positive Coombs' test, periarteritis nodosa, antibodies to thyroid and heart muscle, and clinical pancarditis and thyrotoxicosis. The blood from the proband's mother was strongly Coombs' and ANA positive. In another family, all the members with identifiable serological changes and evidence of AIHD had parents who both demonstrated serological abnormalities of AIHD. In this case, a recessive pattern of inheritance was proposed.

With respect to immune-mediated thrombocytopenia, there has been a strong association with the presence of an alloantigen of the HLA-D locus (called DRw2) and the genetic predisposition to this disease (Karpatkin, 1980). The DRw2 alloantigen was found in 75% of 20 consecutive patients in the New York City area with immune thrombocytopenia, whereas it was present in only 23% of an ethnically matched healthy control population. Of additional interest has been a parallel association of the DRw2 and DRw3 alloantigens of the major histocompatibility complex in patients with SLE (Karpatkin, 1980).

The most convincing evidence of genetic transmission of AIHD in dogs has involved the studies by Lewis and colleagues on a colony of animals with SLE (Lewis *et al.*, 1965, 1973; Schwartz, 1975). Since then, the author has investigated several dog families with what appears to be a familial predisposition to immune-mediated hematological diseases. The cumulative data are shown in Figs. 1 and 2, and Table III. In addition, two 1-year-old littermate cats were reported to have a Coombs'-positive, feline leukemia virus-negative hemolytic anemia (Utroska, 1980).

E. Relationship to Viral Infections and Vaccinations

1. Viral Infections

Many diseases of immune-mediated origin are associated with or triggered by virus infections (Dacie, 1967; Williams *et al.*, 1972; Lewis *et al.*, 1973; Tizard, 1977; Karpatkin, 1980). Viruses that infect lymphoid tissues are apparently capable of interfering with immunological control, thus leading to production of antibodies directed against self-antigens. This relationship has been the most clearly established for SLE.

The pathogenesis of SLE in humans and animals involves environmental influences, drugs such as procainamide, hydralazine, isoniazid, trimethadione, and primidone, and genetic predisposition (discussed in Section V,D), as well as viruses (Tizard, 1977; Halliwell, 1978). In the NZB/NZW mouse hybrid predisposed to develop SLE, breeding experiments suggested direct genetic transmission (Bielschowsky *et al.*, 1959), whereas infection with type C virus particles induced serological changes in other laboratory mice (Mellors, 1969). Type C virus-infected mice developed autoantibodies against nucleic acids and erythrocytes. In the dog, either type C or paromyxovirus (human measles or canine distemper viruses) infections have been associated with SLE (Lewis *et al.*, 1973; Schwartz, 1975). In experimental studies when SLE-affected dogs were bred, the number of serologically affected progeny was higher than could be accounted for by genetic influences alone (Lewis *et al.*, 1973). These findings suggested that vertical transmission was also involved. In other studies, when cell-free filtrates from healthy, LE-cell–positive dogs were administered to newborn mice and puppies, similar serological abnormalities were produced. However, despite the presence of a high incidence of ANA and positive LE preparations, neither the SLE-affected dogs nor those infected with virus developed clinical signs of SLE. Thus the question of the communicable nature of canine SLE is unresolved (Halliwell, 1978).

In humans a similar viral association has been proposed because individuals with SLE frequently have high titers to parainfluenza 1 and measles viruses (Tizard, 1977). Furthermore, myxovirus nucleoprotein strands have been observed in the endothelial cells of kidney biopsy specimens taken from SLE patients. With respect to AIHD in humans, several virus diseases, particularly infectious mononucleosis and mycoplasmal pneumonia, have been encountered (Dacie, 1967).

Acute-onset thrombocytopenic purpura in children is usually preceded by a seasonal (winter or spring) viral illness 1–3 weeks beforehand (Lusher and Iyer, 1977; Karpatkin, 1980). The most commonly associated viruses are varicella, rubella, rubeola, and pharyngitis. In a study of 305 children with idiopathic thrombocytopenic purpura, 80% had an antecedent viral infection (Luscher and Iyer, 1977). It has been postulated that the platelet membrane is altered by the virus or by soluble viral antigen–antibody complexes with an affinity for sites on

the platelet surface. The platelets are thus susceptible to rapid destruction in the spleen or other parts of the reticuloendothelial system. Both sexes are equally affected in this situation, and the disease has an average duration of 1 to 2 months. From 7 to 28% of such children will subsequently develop a chronic thrombocytopenia syndrome. The chronic form of thrombocytopenia is more common in adults and usually occurs in females. Remissions in clinical signs occur, although the platelet count remains consistently at one-third to one-half of normal values (Karpatkin, 1980). Some patients have an intermittent form of thrombocytopenia with cyclical episodes occurring at 3- to 6-month intervals. The relationship of viral diseases to these latter two forms of thrombocytopenia is unclear.

2. *Vaccinations*

Recent vaccinations against viral diseases have been implicated as causes of acute-onset thrombocytopenia in children (Oski and Naiman, 1966; Bachand *et al.*, 1967; Lusher and Iyer, 1977; Karpatkin, 1980). The effect is seen during the period of viremia, 5–10 days after vaccination. Measles vaccine has been reported most frequently to cause such reactions (Oski and Naiman, 1966; Lusher and Iyer, 1977). Experimental studies in a variety of domestic and wild animals (bear, deer, fox, raccoon) have shown a consistent drop of about 100,000 per mm^3 in the circulating platelet count, beginning 3–5 days following use of modified live measles or canine distemper vaccines and lasting for as long as a week thereafter (Dodds, 1982). In clinical situations, a number of cases of bleeding tendency with petechiation and ecchymotic hemorrhages of the skin and mucous membranes have been reported following routine use of modified live measles and distemper virus vaccines in dogs (Dodds, 1980), and hog cholera vaccine in swine (Pilchard, 1966). In addition, animals with a preexisting congenital or hereditary hemostatic defect, such as hemophilia or von Willebrand's disease, frequently exhibit clinical signs of a bleeding tendency in the 5- to 10-day postvaccinal period. Elective surgery is thus contraindicated during this interval (Dodds, 1980).

Whether the vaccine-induced thrombocytopenia and thrombopathy are caused by an immunological mechanism is unknown, but many investigators and clinicians support this concept (Lusher and Iyer, 1977). Because the effect is not seen prior to the viremic phase postvaccination, one could postulate a hapten-mediated immune mechanism inwhich antibodies develop to the viral-coated or virus-infected cell. Immunofluorescent studies of the bone marrow of animals 7–10 days after viral infection or vaccination have located viral particles within megakaryocytes (Osborn and Shahidi, 1973), and megakaryocyte involvement and/or damage from viral infections have been implicated in the purpura associated with childhood varicella (Lusher and Iyer, 1977). Parallel studies of experimentally induced *Ehrlichia canis* infection in dogs have demonstrated increased platelet destruction by an immunological process primarily involving the spleen (Smith *et al.*, 1975).

The situations just described implicate vaccination with attenuated live viruses as one of the causative factors or "triggers" of immune-mediated disease in a susceptible host. That these adverse reactions can also occur in families provides evidence in support of genetic predisposition as an etiological factor. Although speculative at this point, the possibility exists that frequent exposure to monovalent or multivalent modified live vaccines sensitizes a susceptible host to viral antigen and increases the risk of developing immune-mediated reactions. There is a trend among virologists and immunologists to be cautious about the indiscriminate use of live virus vaccines, especially because of the risk of mutation or reversion of shed virus in vaccinated individuals to a virulent form. For example, could the current endemic of gastroenteric virus diseases of dogs (corona and parvoviruses) have resulted from mutated strains of vaccine viruses from this or other species? More specifically, could a mutant strain of cat enteritis (feline panleukopenia) virus have produced the highly infectious, virulent canine parvovirus (CPV)? In addition to the potential for viral reversion to a pathogenic form, mutated viruses could be recognized as new, foreign antigens to the host population, and the increased frequency of immune-mediated anemias and thrombocytopenias might thus be associated with the recent and concomitant widespread exposure to and vaccination against CPV.

Since the beginning of 1981, our laboratory and several other veterinary clinical pathology laboratories have been compiling careful histories of cases referred for immune-mediated blood diseases. In many cases there has been an association with recent exposure to CPV disease or vaccination 1 day to 3 weeks previously with modified live virus (MLV), feline panleukopenia virus (FPV), or CPV vaccine to protect against CPV disease (Young, 1981; Dodds, 1982; C. Pertz, 1981, personal communication; A. G. Ibsen, 1982, personal communication). In only a few instances did the recent history include vaccination with the killed form of FPV or CPV vaccine. A high percentage of these cases have been severe and resulted in permanent side effects, poor prognosis, or death. Some cases of immune-mediated anemia have progressed to a fatal dyplastic anemia-like syndrome whereby the marrow ceases to produce new red blood cells and/or platelets. In other recovered cases, the marrow remains dyplastic for about 3 weeks, after

which there is a rapid, dramatic erythrogenic response, which behaves as though some "toxic" marrow suppression were suddenly lifted. Reticulocyte counts rebound from zero to over 20% of the blood smear in a matter of 3 to 4 days, once the marrow begins to respond (Dodds, 1982). This very serious syndrome has been particularly common in the Old English sheepdog, and several of these affected dogs have been younger than is usually encountered in immune-mediated disease (<2 years old) and were males. Nine affected sheepdogs had the same sire (Table III), and four were from the same litter of seven that had initial signs of disease from 5 to 10 months of age. Three of these dogs died as a result of nonresponsive erythroid dysplasia. In the Scottish terriers referred for immune-mediated anemia and thrombocytopenia (Section V,C), signs of disease in most cases followed recent vaccination with MLV, FPV, or CPV. Two 8-week-old golden retriever littermates were referred for profound anemia and jaundice. Both had strongly positive Coombs' tests directed against IgG + complement, and both responded dramatically to high doses of dexamethasone. Both pups had received MLV distemper–hepatitis–parainfluenza–CPV vaccinations 6 days previously. Another case involved a healthy 4-year-old poodle given a routine MLV–CPV vaccination. Within 24 hr the animal's skin and mucous membranes were covered with tiny purple bruises, and the platelet count was <10,000 per mm^3. The dog recovered uneventfully following aggressive treatment with dexamethasone.

Perhaps the most bizarre reactions observed to follow vaccination with MLV–FPV or –CPV vaccines have been pemphigus-like disorders. In six cases, the toenails on all four feet began to slough in a painless, nonswollen manner 7–10 days after vaccination (Dodds, 1982; C. Pertz, 1981, personal communication). All toenails eventually sloughed and had not regrown as of this writing. A widespread necrotizing dermatitis of the decubital areas of the limbs was reported in two litters of German shepherd puppies from the same sire (Ibsen, 1982). Both litters had been vaccinated 2 weeks previously with a multivalent product containing MLV–CPV. The third unusual finding has been an increase in the number of dogs referred with severe icterus, tender abdomens, swollen livers, and markedly elevated hepatic serum enzymes (Dodds, 1982; R. J. Wilkins and A. I. Hurvitz, 1981, personal communication; S. Crowe, 1982, personal communication). The history of such cases with a hepatitis-like syndrome has usually been a recent exposure to active cases of CPV disease or MLV–FPV or –CPV vaccination in the previous 1–4 weeks.

It cannot be concluded from these observations that MLV–FPV or –CPV vaccines are unsafe for dogs for several reasons. First, hundreds of thousands of dogs have been routinely and repeatedly vaccinated for

CPV with the feline and canine MLV and killed vaccines without obvious side effects. Thus the relatively few cases of documented or apparent reactions may have involved only those with a susceptible genetic or physiological makeup. Unfortunately, there is no way to predict or identify susceptible individuals. Avoiding the repeated use of MLV–FPV, or –CPV vaccines for close relatives of known immune reactors is one way to reduce the risk of immune-mediated problems. Such dogs should, of course, be given regular, spaced immunizations with the killed feline- or canine-origin vaccines to protect against overt CPV disease. Second, the fact that the observed reactions have frequently included a recent history of MLV, FPV, or CPV vaccination may be coincidental and not causally related for many of the cases. In a few situations, however, an immediate, severe febrile and immune-mediated destruction of red blood cells and/or platelets has followed vaccination within 24 to 48 hr. These reactions clearly were vaccine related, but it cannot be concluded that MLV, FPV, or CPV vaccine per se was the cause, because a similar reaction might have occurred if the dog had received another type of vaccine. Third, could the observed reactions result from the frequent revaccinations given to produce protective titers in relatively nonresponsive animals? Repeated exposure to viral antigen by frequent vaccinations could sensitize the host and eventually cause an immune-mediated reaction. In such dogs, what is a "safe" interval between vaccinations?

In summary, further research is necessary on the immune responses of dogs to vaccination procedures that would normally be considered to be routine. Whether immune-mediated reactions occur only in genetically predisposed or susceptible individuals needs to be clarified. Alternatively, certain types of viral antigens, such as CPV or FPV, may be more likely to trigger immune-mediated reactions. In this case, MLV vaccines may produce a severe immune reaction as the virus multiplies in the host and provides more antigenic stimulation. However, on this basis MLV vaccines produce higher antibody titers than killed vaccines, a factor that is beneficial to the host. The advantages and possible risks involved must be considered before a decision is made about the type and frequency of vaccines to be used. It is important that the veterinary profession record any usual drug or vaccine complications, especially those that produce immunological reactions.

F. Hormonal Influences

Traditionally, immune-mediated diseases are two to three times more common in females than in males, and this trend also applies to animals whether they are intact or neutered (Schalm, 1975; Dodds,

1977; Tizard, 1977; Halliwell, 1978). Our current data (Table III and Fig. 1) also support this sex predisposition. In acute idiopathic thrombocytopenic purpura of childhood, there is no sex difference (Lusher and Iyer, 1977; Karpatkin, 1980), but whether this applies to the similar syndrome in young animals is unknown. An interesting finding in the 305 cases of acute thrombocytopenia in childhood reviewed by Lusher and Iyer (1977) was an 87% preponderance in whites, with only 13% of black racial origin, although the pediatric hospital population at large was 55% black. These authors speculated that the less severe form of the disease with superficial petechiae and bruises might go unnoticed by the parents of a black child. However, only one child had a chronic, mild form of disease, thus a real difference in racial susceptibility was likely. A similar situation pertains in animals, especially of the poodle breed, in which acute-onset thrombocytopenic purpura is much more commonly seen in white, light-skinned animals than in grey or black dogs (Wilkins *et al.*, 1973).

That the frequency of AIHD is similar in intact or spayed females is difficult to reconcile with the concept that hormonal influences are important in triggering or predisposing individuals to immune-mediated disease. Certainly our findings with the vizsla family (Fig. 1) strongly suggest an association between reproductive irregularities and the subsequent appearance of antierythrocyte and/or antiplatelet antibodies and overt hematological disease or the risk of such a disease.

Stress situations including pregnancy (Williams *et al.*, 1972; Schalm, 1975; Dodds, 1977); hormonal imbalance such as abnormal or irregular estrous cycles, pyometra, and pseudocyesis (Fig. 1, Table IV; Dodds, 1977); and other underlying diseases precipitate episodes of disease in early or subclinical cases, and aggravate the preexisting disease of affected individuals. In our experience, spaying females in these instances has averted overt clinical disease.

G. Drug Reactions

Drug-induced immune hemolysis and/or thrombocytopenia occurs in humans and animals (Williams *et al.*, 1972; Wilkins *et al.*, 1973; Dodds, 1977; Schoen and Trentham, 1981). Three mechanisms have been proposed to account for the majority of these conditions: a haptene mechanism, immune-complex formation, and true autoantibody induction (Dodds, 1977). Schoen and Trentham (1981) have challenged the traditional theories and proposed that, for drug-induced SLE at least, the drug acts as an adjuvant or immunostimulant to trigger polyclonal B- and T-lymphocyte activation and immune dysregulation; this would explain the widespread disruption of self-tolerance observed. They cite as evidence in support of the concept that experimental animal models of autoimmunity can readily be induced by injections of a variety of tissue components in complete Freund's adjuvant, thus producing allergic encephalomyelitis, thyroiditis, orchitis, uveitis, and polyarthritis (Schoen and Trentham, 1981).

The therapeutic agents implicated in provoking SLE in humans include hydralazine, procainamide, isoniazid, practolol, hydantoins, chlorpromazine, D-penicillamine, and nitrofurantoin (Schoen and Trentham, 1981). Other drugs that can produce AIHD and/or thrombocytopenia are quinine, quinidine, stibophen, sedormid, α-methyldopa, cephalothin, indomethacin, phenacetin, phenylbutazone, dilantin, streptomycin, and the most commonly recognized causative drugs, penicillin and heparin (Babcock *et al.*, 1976; Dodds, 1977). Several of these drugs also produce a parallel disease entity in animals. It is generally believed that drug reactions of this type occur only in genetically susceptible subjects. An increased risk for the development of drug-induced SLE has been reported in persons with the alloantigen HLA-DRw4 (Schoen and Trentham, 1981).

Heparin-induced thrombocytopenia is a commonly reported disease in humans (Babcock *et al.*, 1976). An immunological mechanism has been proposed for this effect in some patients, whereas in others and particularly in species such as the dog, hamster, rat, and guinea pig (Babcock *et al.*, 1976; Dodds, 1980), heparin induces platelet aggregation *in vivo*, which causes thrombocytopenia.

H. Stress and Underlying Disease

Immune-mediated diseases are associated with a variety of underlying diseases and are frequently precipitated by stress (environmental and hormonal influences) in genetically susceptible individuals (Dacie, 1967; Williams *et al.*, 1972; Dodds, 1977). The most commonly associated diseases in humans and animals are lymphoreticular malignancies, especially lymphocytic leukemia, lymphosarcoma, and reticulum cell sarcoma, and other autoimmune disorders such as SLE, rheumatoid arthritis, and immune-mediated thrombocytopenias. Rarely, cases have occurred with carcinomas, viral diseases such as infectious mononucleosis, mycoplasmal pneumonia, severe bacterial infections, and inflammatory or granulomatous diseases (ulcerative colitis, rheumatic fever, acute and chronic liver disease, and sarcoidosis) (Dodds, 1977).

Extremes in temperature result in seasonal occurrences of AIHD and/or thrombocytopenia (Karpatkin, 1980). In our experience, chronic cases of canine AIHD or thrombocytopenia in remission or under control frequently relapse a few days to a week following a severe cold spell in winter or hot spell in summer (Dodds, 1982). Five dogs with chronic AIHD had a sudden relapse with *in vivo* hemagglutination and hemolysis 2–3 days following extremely hot weather. The blood of these animals had been monitored on a regular basis by laboratory testing for more than a year previously, and had been negative for serological evidence of antierythrocyte activity. All five dogs relapsed at the same time and were referred to our laboratory by three different veterinary clinics. This was a dramatic example of the effects of environmental stress in aggravating preexisting disease.

VI. Management and Treatment

Management of acute cases of immune-mediated anemia and/or thrombocytopenia consists mainly of reducing stress and restricting activity to reduce the chance of trauma, especially to the head. Although intracranial hemorrhage is a relatively rare complication, in severe thrombocytopenia bleeding into the central nervous system is a serious and often fatal occurrence.

Treatment should be directed at correcting the anemia and keeping the patient free of purpura, if thrombocytopenia is present. It is not necessary to restore the platelet count to normal for counts of 50,000 to 80,000 per mm^3 are usually sufficient in humans and animals (Lusher and Iyer, 1977; Dodds, 1980; Karpatkin, 1980) to prevent bleeding.

A. Red Cell Disorders

With respect to AIHD, transfusions should be avoided whenever possible because they accelerate hemolysis (Dacie, 1967; Williams *et al.*, 1972; Schalm, 1975; Dodds, 1977; Pearson, 1980). Individuals with chronic, compensated anemia can tolerate low hematocrits (12–20%) quite well and can be managed in a nonstressed environment without transfusions. If replacement is essential, it is crucial that transfusions be with typed, cross-matched red cells (Dodds, 1977). Truly serocompatible blood does not exist for these patients, and so the transfused cells will have a shortened *in vivo* survival.

The treatment of choice for immune-mediated anemias is corticosteroids (Williams *et al.*, 1972; Schalm, 1975; Schalm *et al.*, 1975; Dodds, 1977). The effect is quite rapid (24–48 hr); the mechanism

whereby steroids ameliorate AIHD is unknown but may involve suppression of erythrophagocytosis as well as the immune response. Severely affected patients need high doses of parenteral steroids until the hematocrit has stabilized, followed by oral medication at reduced levels once the hematocrit starts to rise and the reticulocyte count falls. Maintenance doses of steroids are given every other day for the next 1–2 months. In some patients, low maintenance doses are required on a long-term basis to prevent relapses. Only minor side effects such as weight gain have been encountered with long-term treatment (2–3 years) in dogs (Dodds, 1982). In humans, about 75% of cases improve and/or stabilize even if they remain Coombs' positive, and only about 5–10% of cases are refractory to steroids. It is not advisable to treat Coombs'-positive cases that have no clinical signs with steroids.

In our collective experience with over 300 cases, the steroid of choice, especially in severe disease, is dexamethasone. In the rare but near-fatal cases of erythroid dysplasia syndrome mentioned earlier (Section V,E), aggressive therapy with dexamethasone was the only treatment to which the patients responded. Why this corticosteroid appears to be more efficacious that the commonly used prednisone or prednisolone is unknown, although we may now be encountering immune-mediated disorders of different etiology than were seen previously.

Our dosage of dexamethasone is calculated as follows: body weight divided by eight and given as milligrams of dexamethasone on a daily basis. This dosage is given in divided doses two to three times a day. For example, a 40-pound (18.4-kg) dog would receive a total of 5 mg dexamethasone daily as 2, 1, and 2 mg during the course of 24 hr. This amount is continued for 5 to 7 days and is roughly equivalent to 1 mg/pound/day of prednisone. The dosage is then reduced by one-third to one-half for another week and then again by one-third to one-half for the third week. After this time, treatment is maintained at 0.03 to 0.05 mg/pound/day every other day, as needed. Contrary to popular and pharmacological opinion, alternate-day dexamethasone treatment has worked well for our cases as well as for other clinicians. In some cases, veterinarians have switched their patients from dexamethasone to prednisone for long-term maintenance. In our experience, it probably is not important which steroid is used for most patients once the disease process is under control, but we do have a group of patients in which relapses occur within a week of discontinuing dexamethasone therapy and/or switching to prednisone or prednisolone. We therefore recommend maintaining dexamethasone as the steroid of choice as long as it is needed. Side effects of long-term therapy have not been a significant problem.

Splenectomy may be necessary or useful in steroid-refractory cases,

in patients with frequent relapses, or when steroids are required continuously in high doses to maintain the patient. The use of splenectomy is still controversial, however, and recently has been called an obsolete concept for routine treatment (Pearson, 1980). Once splenectomy is performed, the patient is at risk for severe infection (especially pneumococcal) and rapid death from a disseminated intravascular coagulation syndrome. This postsplenectomy syndrome is uncommon in adults but is of concern in children, especially those with an underlying associated primary disease (Jiji *et al.*, 1973; Lusher and Iyer, 1977; Karpatkin, 1980; Pearson, 1980). Whether this syndrome occurs in animals is unknown, but it should be kept in mind that splenectomized animals are at risk to develop hemobartonellosis (Schalm *et al.*, 1975). In our experience, splenectomy has not been necessary in the management of chronic AIHD. The most important factor in successful management has been the immediate initiation of an appropriate steroid regimen.

Antimetabolite drugs or irradiation are also being used to treat AIHD in humans and animals. This treatment is reserved for cases that are steroid resistant, require very high doses of steroids, and/or did not respond to splenectomy. Use of such drugs requires careful monitoring of the patient because of the possibility of toxic side effects. The most commonly used drugs are the vinka alkaloids (Vincristine, vinblastine), cyclophosphamide, imuran, and 6-mercaptopurine (Dodds, 1977; Lusher and Iyer, 1977; Halliwell, 1978; Kelton *et al.*, 1981).

B. Platelet Disorders

The treatment of immune-mediated thrombocytopenia is basically the same as that discussed and recommended (Section VI,A) for red cell disorders. We have found dexamethasone to be consistently more successful in reversing thrombocytopenia than prednisone or prednisolone. If this treatment is to succeed, patients must be treated as soon as thrombocytopenia is discovered and not after several days or weeks of intermittent treatment with a variety of drugs or transfusions. Cases refractory to prednisone or prednisolone are usually responsive to aggressive dexamethasone therapy, but it takes higher doses for more extended periods than would have been needed if dexamethasone had been used at first. The more debilitated the patient and the more chronic the disease, the more difficult it will be to reverse the process with steroid therapy alone. Long-term maintenance with alternate-day dexamethasone has successfully controlled immune

thrombocytopenia in dogs, cats, and horses, whereas prednisone has not been as effective in another series of cases (Dodds, 1982). As mentioned earlier, there is little value in bringing the platelet count to normal values with steroid therapy (Karpatkin, 1980). Bleeding is unlikely to occur with platelet counts above 50,000 to 80,000 per mm^3, provided that the available cells are functional. We aim with our animal patients to keep platelet counts around 100,000 per mm^3, because higher counts usually require doses of steroids, which place the patient at risk for hypercorticism.

Curiously, in acute childhood purpura, use of steroids is not routine other than for the first few days after onset, and, according to several experienced investigators (Lusher and Iyer, 1977; Karpatkin, 1980), there is no real evidence that they are of benefit in reducing the risk of serious complications such as intracranial hemorrhage. Perhaps the differing experience with thrombocytopenia in animals reflects the fact that most cases are not in neonates or young animals and represent models of the chronic thrombocytopenias of humans.

The case for splenectomy in acute and chronic immune thrombocytopenia in humans is more justified than in AIHD, although this is also controversial (Jiji *et al.*, 1973; Lusher and Iyer, 1977; Karpatkin, 1980: Pearson, 1980). Certainly the majority of patients respond to corticosteroids, splenectomy, immunosuppressive therapy, or a combination thereof (Kelton *et al.*, 1981). About 5 to 10% of childhood thrombocytopenias become chronic, and these respond best to splenectomy (Lusher and Iyer, 1977). A favorable response to moderate doses of steroids usually indicates that the spleen is the major site of platelet destruction, which suggests that the patient will benefit from splenectomy (Karpatkin, 1980). Splenectomy has been reported to be successful in 100% of such patients but has also been useful in 79% of patients who were refractory to steroids. The rationale for splenectomy is that it removes the potential site of platelet destruction as well as a major source of antiplatelet antibody production, and it restores to the body the active platelet pool (about 40%) normally sequestered in the spleen. Interestingly, the antiplatelet antibody frequently persists afterwards despite the apparent clinical remission (Karpatkin, 1980). In patients with severe thrombocytopenia the liver is also a major source of platelet destruction, and so patients who fail to respond to splenectomy may be exhibiting hepatic sequestration of platelets. The risk of infection and death postsplenectomy, especially in children, should be kept in mind (Jiji *et al.*, 1973; Lusher and Iyer, 1977; Karpatkin, 1980; Pearson, 1980).

Despite the conclusions of many investigators concerning the bene-

fits of splenectomy for immune thrombocytopenia in humans (Lusher and Iyer, 1977; Karpatkin, 1980) and animals (Halliwell, 1978), we have resorted to splenectomy in only 2 cases of the more than 200 studied (Dodds and Wilkins, 1977). Neither case responded to splenectomy. We also have not used antimetabolite drugs for these cases, although one problem case was nonresponsive to high doses of dexamethasone alone and responded to two, weekly spaced injections of vincristine along with the dexamethasone (Dodds, 1982). Use of antimetabolite drugs in humans and animals with thrombocytopenia has been increasing, and our recommendations are that clinicians rely on treatment regimen(s) that have been successful in their own experience.

Platelet transfusions with fresh platelet-rich plasma or fresh or frozen platelet concentrates have a very limited role in the management of acute thrombocytopenia of childhood (Lusher and Iyer, 1977). Usually such transfusions neither alleviate bleeding nor produce a detectable rise in platelet count, and the transfused platelets are rapidly destroyed. Therefore, platelet transfusions are reserved for cases of life-threatening bleeding or to cover the patients for surgical procedures. Occasionally platelet transfusions are needed in conjunction with high doses of steroids to protect the patient during splenectomy.

To summarize the information available for treatment of thrombocytopenia (Karpatkin, 1980), about 50% of patients respond to steroids alone. Long-term therapy is usually required because cessation results in eventual relapse in chronic cases. Splenectomy is successful in about 65 to 70% of human cases, but has not been needed in our experience with over 200 cases in dogs. Patients refractory to steroids and splenectomy present more serious problems. About a third of these are also refractory to immunosuppressive therapy and have frequent relapses.

VII. Prevention

Unfortunately, until affected patients are admitted with clinical signs of immune-mediated disease, there is no way to identify those at risk in the population at large. In families with an apparent genetic predisposition to such diseases, however, serial monitoring of the relatives, as discussed earlier (Section III,C), can be helpful in predicting eventual disease. It is important to remember that it is not uncommon for clinically healthy relatives of affected individuals to have positive

serological evidence of AIHD and/or thrombocytopenia without disease. Thus, a safeguard for all breeding stock, especially females with reproductive irregularities, is to monitor them on a regular basis for serological changes compatible with impending immune-mediated disease. This approach has been used successfully in our laboratory for the past 5–6 years with several affected families of dogs (Figs. 1 and 2) (Dodds, 1982).

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References

Babcock, R. B., Dumper, C. W., and Scharfman, W. B. (1976). *N. Engl. J. Med.* 295, 237–241.
Bachand, A. J., Rubenstein, J., and Morrison, A. N. (1967). *A. J. Dis. Child.* 113, 283–285.
Bielschowsky, M., Helyer, B. J., and Howie, J. B. (1959). *Proc. Univ. Otago Med. Sch.* 37, 9–14.
Branchog, I. (1975). *Br. J. Haematol.* 29, 413–426.
Byars, T. D., and Greene, C. E. (1982). *J. Am. Vet. Med. Assoc.* 180, 1422–1424.
Cines, D. B., and Schreiber, A. D. (1979). *N. Engl. J. Med.* 300, 106–111.
Dacie, J. V. (1967). "The Haemolytic Anemias, Congenital and Acquired," 2nd ed. Grune & Stratton, New York.
Dacie, J. V., and Worlledge, S. M. (1968). *Prog. Hematol.* 6, 82–120.
Dobbs, C. D. (1965). *Arch. Intern. Med.* 116, 273–276.
Dodds, W. J. (1977). *J. Am. Anim. Hosp. Assoc.* 13, 437–441.
Dodds, W. J. (1980). *In* "Clinical Biochemistry of Domestic Animals" (J. J. Kaneko, ed.), 3rd ed., pp. 701–712. Academic Press, New York.
Dodds, W. J. (1982). New York State Department of Health, Albany (unpublished data).
Dodds, W. J., and Wilkins, R. J. (1977). *Am. J. Pathol.* 86, 489–491.
Duquesnoy, R. J., Lorentzen, D. F., and Aster, R. (1975). *Blood* 45, 741–747.
Fialkow, P. J. (1964). *Am. J. Med.* 36, 188–199.
Forget, B. G. (1980). *Hosp. Pract.* 15, 67–78.
Gengozian, N., and Rice, D. T. (1982). *Clin. Exp. Immunol.* 47, 431–434.
Halliwell, R. E. W. (1978). *Adv. Vet. Sci. Comp. Med.* 22, 221–263.
Hirschman, R. J., Gralnick, H. R., and Schaff, F. (1974). *J. Lab. Clin. Med.* 84, 292–297.
Hymes, K. H., Shulman, S., and Karpatkin, S. (1979). *J. Lab. Clin. Med.* 94, 639–648.
Jain, N. C., and Kono, C. S. (1980). *Vet. Clin. Pathol.* 9, 10–14.
Jiji, R. M., Firozvi, T., and Spurling, C. L. (1973). *Arch. Intern. Med.* 132, 380–383.
Joshi, B. C., and Jain, N. C. (1976). *Am. J. Vet. Res.* 37, 681–685.

Karpatkin, S. (1980). *Blood* 56, 329–343.
Karpatkin, S., Strick, N., Karpatkin, M. B., and Siskind, G. W. (1972). *Am. J. Med.* 52, 776–784.
Kelton, J. G., McDonald, J. W. D., Barr, R. M., Walker, I., Nicholson, W., Neame, P. B., Hamid, C., Wong, T. Y., and Hirsh, J. (1981). *Blood* 57, 431–436.
Lewis, R. M., Schwartz, R., and Henry, W. B. (1965). *Blood* 23, 143–160.
Lewis, R. M., Andre-Schwartz, J., Harris, G. S., Hirsch, M. S., Black, R. H., and Schwartz, R. S. (1973). *J. Clin. Invest.* 52, 1893–1907.
Lusher, J. M., and Iyer, R. (1977). *Semin. Thromb. Hemostasis* 3, 175–199.
Mellors, R. C. (1969). *J. Infect. Dis.* 120, 480–487.
Morse, B. S., Giuliani, D., and Nussbaum, M. (1981). *Blood* 57, 809–811.
Morse, B. S., Giuliani, D., and Nussbaum, M. (1982). *Am. J. Hematol.* 12, 271–275.
Myers, T. J., Kim, B. K., Steiner, M., Bishop, J., and Baldini, M. G. (1981). *J. Lab. Clin. Med.* 97, 854–863.
Newton, C. D., Lipowitz, A. J., Halliwell, R. E. W., Allen, H. L., Biery, D. N., and Schumacher, R. (1976). *J. Am. Vet. Med. Assoc.* 160, 113–121.
Osborn, J. E., and Shahidi, N. T. (1973). *J. Lab. Clin. Med.* 81, 53–63.
Osborne, C. A., Stevens, J. R., McClean, R., and Vernier, R. L. (1973). *J. Am. Anim. Hosp. Assoc.* 9, 295–300.
Oski, F. A., and Naiman, J. K. (1966). *N. Engl. J. Med.* 275, 352–356.
Pearson, H. A. (1980). *Hosp. Pract.* 15, 85–94.
Pedersen, N. C., Pool, R. C., Castles, J. J., and Weisner, K. (1976). *J. Am. Vet. Med. Assoc.* 169, 295–303.
Pilchard, E. I. (1966). *J. Am. Vet. Med. Assoc.* 148, 48–51.
Robbins, J. B., Skinner, R. G., and Pearson, H. A. (1969). *N. Engl. J. Med.* 280, 75–79.
Schalm, O. W. (1975). *Can. Pract.* 2, 37–45.
Schalm, O. W., Jain, N. C., and Carroll, E. J. (1975). "Veterinary Hematology," 3rd ed., p. 644. Lea & Febiger, Philadelphia, Pennsylvania.
Schoen, R. T., and Trentham, D. E. (1981). *Am. J. Med.* 71, 5–8.
Schultz, R. D. (1976). "Laboratory Diagnosis of Immunologic Disorders in the Dog and Cat." Miles Laboratories, Research Products, Elkhart, Indiana.
Schwartz, R. S. (1975). *N. Engl. J. Med.* 293, 132–136.
Shebani, O. I., and Jain, N. C. (1983). *Vet. Clin. Pathol.* 11, 23 (abstr.).
Slappendel, R. J. (1979). *Vet. Immunol. Immunopathol.* 1, 49–59.
Slappendel, R. J., van Erp, C. L. G. M., Goudswaard, J., and Bethlehem, M. (1970). *Tijdschr. Diergeneeskd.* 100, 445–460.
Smith, R. D., Ristic, M., Huxsoll, D. L., and Baylor, R. A. (1975). *Infect. Immun.* 11, 1216–1221.
Sugiura, K., Steiner, M., and Baldini, M. G. (1980). *J. Lab. Clin. Med.* 96, 640–653.
Tizard, I. R. (1977). *In* "Introduction to Veterinary Immunology," pp. 169–183. 279–288, 312–327. Saunders, Philadelphia, Pennsylvania.
Tizard, I. R. (1978). *Bi-Week. Small Anim. Vet. Med. Update Ser.* 16, 1–8.
Utroska, B. (1980). *VM/SAC, Vet. Med. Small Anim. Clin.* 11, 1699–1701.
Verheyden, C. N., Beart, R. W., Jr., Clifton, M. D., and Phyliky, R. L. (1978). *Mayo Clin. Proc.* 53, 442–446.
Videback, A. (1962). *Acta Med. Scand.* 171, 187–194, 449–462.
Wilkins, R. J., Hurvitz, A. I., and Dodds-Luffin, W. J. (1973). *J. Am. Vet. Med. Assoc.* 163, 277–282.
Williams, W. J., Beutler, E., Erslev, A. J., and Rundles, R. W., eds. (1972). "Hematology," pp. 486–510. McGraw-Hill, New York.