Intravenous administration of human immune globulin in dogs with immune-mediated hemolytic anemia

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Objective—To evaluate the efficacy and safety of intravenous administration of human immune globulin in the treatment of dogs with immune-mediated hemolytic anemia (IMHA).

Design—Prospective clinical trial.

Animals—10 dogs with confirmed primary IMHA that had failed to respond to conventional immunosuppressive treatment (administration of prednisone and cyclophosphamide or azathioprine).

Procedure—Diagnosis of IMHA was confirmed by detecting spherocytosis or autoagglutination in blood smears and by excluding secondary causes of IMHA. Dogs were treated with human immune globulin (1 g/kg [0.45 g/lb] of body weight, IV) during a 6- to 12-hour period. Prednisone treatment was continued in all dogs, and cyclophosphamide treatment was continued in 4.

Results—Median duration of prior immunosuppressive treatment was 12.5 days. Short-term response could not be evaluated in 2 dogs, because they were given blood transfusions within 7 days after immune globulin treatment. However, there was a significant increase in mean Hct and hemoglobin concentration in 8 other dogs from day 0 to 28 after treatment. Five dogs had clinically meaningful responses to treatment. Three dogs were alive 12 months after treatment. There were not any adverse effects that could be definitively attributed to immune globulin treatment; however, thrombocytopenia was observed in 6 dogs after treatment, and evidence of thromboembolism was detected at necropsy in 5 of the 7 dogs that died.

Clinical Implications—Human immune globulin may be useful for short-term stabilization of some dogs with IMHA; however, it did not appear to improve long-term survival. (*J Am Vet Med Assoc* 1997;210: 1623–1627)

Immune-mediated hemolytic anemia (IMHA) is a common cause of severe anemia in dogs.[1] Despite treatment with high doses of corticosteroids and other cytotoxic drugs such as cyclophosphamide and azathioprine, IMHA may be difficult or impossible to control, and adverse effects associated with drug-induced immunosuppression may be severe. Studies[2-4] in people have found that intravenous administration of immune globulin (**human intravenous immune globulin [hIVIG]**) is efficacious in the treatment of immune-mediated diseases, including IMHA, and the effect of hIVIG in dogs with nonregenerative immune-mediated anemia was recently reported.[5] The purpose of the study reported here was to evaluate the effectiveness and safety of hIVIG treatment in dogs with IMHA that had failed to respond to conventional immunosuppressive treatment.

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Materials and Methods

Ten dogs with primary IMHA that had failed to respond to conventional immunosuppressive treatment with prednisone (2 mg/kg [0.9 mg/lb] of body weight, PO, q 12 h) and cyclophosphamide (200 mg/m^2 of body surface area, IV, q 7 d, in 1 dog; 50 mg/m^2, PO, q 24 h, 4 d/wk, in 4 dogs; and 50 mg/m^2, IV, q 24 h, 4 d/wk, in 1 dog) or azathioprine (2 mg/kg, PO, q 24 h) were included in the study. Diagnosis of primary IMHA had been confirmed by detecting spherocytosis or autoagglutination in blood smears and by excluding secondary causes of IMHA. Diagnostic tests performed on dogs prior to initiation of the study included CBC, platelet and reticulocyte counts, serum biochemical analyses, urinalysis, thoracic and abdominal radiography, cytologic and histologic evaluation of bone marrow, determination of rickettsial titers (*Ehrlichia canis*, *Rickettsia rickettsii*), direct Coombs' test using polyvalent antisera (IgG, IgM, and C3), and determination of antinuclear antibody titer.

All dogs had failed to respond to conventional immunosuppressive treatment. Failure to respond to conventional treatment was defined as not having an increase in Hct after 2 weeks of combination immunosuppressive treatment (prednisone and cyclophosphamide or azathioprine) or having a life-threatening decrease in Hct despite combination immunosuppressive treatment. Human intravenous immune globulin[a] was administered (1 g/kg [0.45 g/lb], IV) during a 6- to 12-hour period. Prednisone administration was continued in all dogs after hIVIG administration, and in 4 dogs, cyclophosphamide administration was also continued. Complete blood, reticulocyte, and platelet counts were repeated on days 1 through 7 and 14, 21, 28, and 42 days after treatment and thereafter as appropriate for clinical management.

To objectively evaluate response to hIVIG treatment, criteria established to determine response in human beings with IMHA were used.[3] A type-I response was defined as an increase in hemoglobin concentration > 2 g/dl within 14 days after hIVIG treatment. A type-II response was defined as an increase in hemoglobin concentration > 2 g/dl and a peak hemoglobin concentration > 10 g/dl within 28 days after hIVIG treatment. Hematocrit and hemoglobin concentration on days 0, 7, 14, 21, and 28 and reticulocyte count on days 0 through 7 were compared by means of ANOVA, followed by a test to determine whether there were linear trends over time.[6,b] A value of $P < 0.05$ was considered significant.

Results

Pretreatment evaluation—Median age and weight of the dogs were 5.5 years (range, 3 to 9 years) and 11

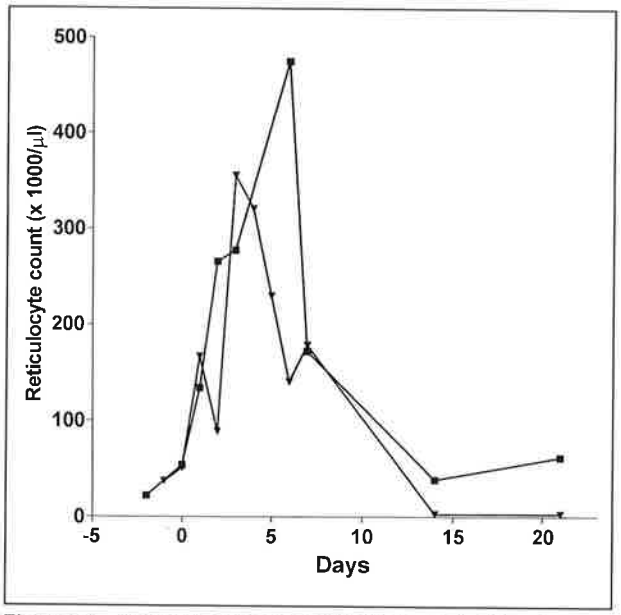

Figure 1—Reticulocyte count before and after human intravenous immune globulin (hIVIG) treatment in 2 dogs with immune-mediated hemolytic anemia (IMHA). Change in reticulocyte count in these 2 dogs is representative of 8 dogs that had an increase in reticulocyte count after hIVIG treatment. Dogs were treated with hIVIG on day 0.

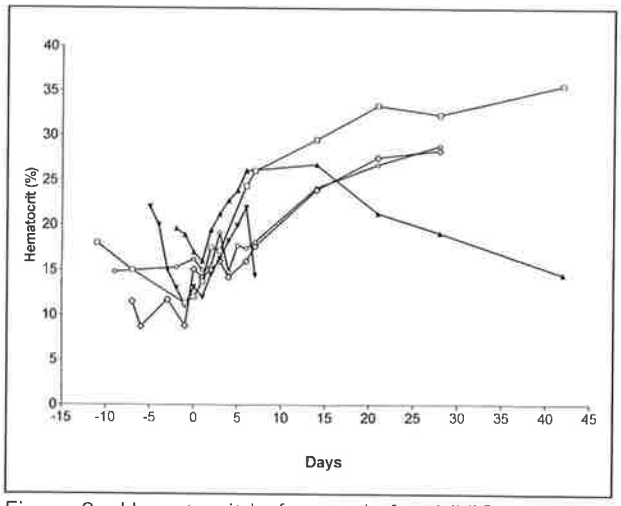

Figure 2—Hematocrit before and after hIVIG treatment in 5 dogs with IMHA that had a type-I (solid symbol) or type-II (open symbol) response to hIVIG treatment. A type-I response was defined as an increase in hemoglobin concentration > 2 g/dl within 14 days after hIVIG treatment. A type-II response was defined as a type-I response and peak hemoglobin concentration > 10 g/dl within 28 days after hIVIG treatment. Dogs were treated with hIVIG on day 0.

kg (24 lb; range, 5 to 47 kg [11 to 104 lb]), respectively. Six dogs were female, and 4 were male. Three were Cocker Spaniels. Six dogs had been treated with prednisone and cyclophosphamide prior to initiation of the study; 2 had been treated with prednisone and azathioprine; 1 had been treated with prednisone, cyclophosphamide, and azathioprine; and 1 had been treated with prednisone, cyclosporine, and azathioprine. Five dogs had received prednisone alone for 3 to 7 days prior to the addition of a cytotoxic drug. Median duration of prednisone treatment prior to entry into the study was 12.5 days (range, 7 to 137 days). Median duration of combination immunosuppressive treatment was 11.5 days (range, 5 to 132 days).

Median Hct immediately prior to hIVIG treatment was 15.5% (range, 12 to 26%). Median hemoglobin concentration was 4.9 g/dl (range, 4.0 to 9.2 g/dl). Median platelet count prior to treatment was 200 × 10^3/µl (range, 37 to 640 × 10^3/µl). Four dogs were thrombocytopenic (< 200 × 10^3/µl). Median reticulocyte count prior to treatment was 53.5 × 10^3/µl (range, 0 to 463 × 10^3/µl). Six dogs had nonresponsive anemia with reticulocyte counts < 60 × 10^3/µl. Spherocytosis was observed in 8 dogs, and autoagglutination was observed in 5. Six dogs had positive Coombs' test results.

Mild to marked neutrophilia was detected in 8 dogs, a left shift was observed in 7, and neutropenia was detected in 1. Eosinopenia was detected in 8 dogs, and lymphopenia was detected in 6. Eight dogs had high alanine aminotransferase activity, 10 had high alkaline phosphatase activity, and 6 had high γ-glutamyltransferase activity. Three dogs had hyperbilirubinemia (range, 1.7 to 7.8 mg/dl), 3 had hypercholesterolemia (range, 303 to 354 mg/dl), and 4 had mild hyperglycemia (range, 133 to 143 mg/dl). All dogs had a low urine specific gravity (< 1.025), and 2 had urinary tract infections (bacteriuria, 2 dogs; positive urine culture results, 1 dog). Abdominal radiography revealed hepatomegaly in 8 dogs and splenomegaly in 3. Thoracic radiography revealed cardiomegaly in 2 dogs and a mild diffuse interstitial pattern in 3. None of the dogs were seropositive for antinuclear antibodies or had serologic evidence of infection by *E canis* or *R rickettsii*. Cytologic and histologic evaluation of bone marrow samples revealed erythroid hyperplasia in all dogs and myeloid hyperplasia in 9.

Response to treatment—In 8 dogs, reticulocyte count increased after treatment (Fig 1). Peak reticulocyte count in these dogs ranged from 167 to 531 × 10^3/µl. In 1 dog, which had moderately regenerative anemia (reticulocyte count, 139 × 10^3/µl), there was no change in reticulocyte count after hIVIG treatment. In the remaining dog, reticulocyte count was high before treatment (464 × 10^3/µl) and decreased after treatment. A significant linear trend was not found in reticulocyte count between days 1 and 7 after treatment.

In regard to change in Hct, 2 dogs had a type-I response, 3 had a type-II response, and 3 did not have a response after hIVIG treatment (Fig 2). Two other dogs required blood transfusions 3 and 7 days after hIVIG treatment, which made it impossible to assess short-term response to hIVIG administration.

Mean Hct prior to treatment for 8 dogs in which short-term response could be evaluated was 16% (median, 14%), and mean hemoglobin concentration was 5.2 g/dl (median, 4.6 g/dl). Mean Hct for 5 dogs that were alive and in which short-term response could be evaluated 28 days after treatment was 27% (median, 28%), and mean hemoglobin concentration was

9.0 g/dl (median, 9.4 g/dl). There was a significant linear trend from day 0 to 28 for mean Hct ($P < 0.0001$) and mean hemoglobin concentration ($P = 0.0001$) in 8 dogs in which short-term response could be evaluated.

In 4 dogs that were thrombocytopenic before hIVIG treatment, platelet count returned to the reference range within 1 to 16 days after treatment; however, it subsequently decreased to less than the reference range again in 3 of the 4 dogs. Of 6 dogs that were not thrombocytopenic before hIVIG treatment, 3 became thrombocytopenic within 1 to 6 days after treatment. Thrombocytopenia was attributed to **disseminated intravascular coagulation (DIC)** in 2 dogs with platelet counts of 33 and $32 \times 10^3/\mu l$. Thrombocytopenia was transient and not associated with clinical evidence of hemorrhage in 3 dogs (lowest platelet counts, 189, 73, and $35 \times 10^3/\mu l$). One dog died while thrombocytopenic (lowest platelet count, $45 \times 10^3/\mu l$); however, the dog was not in DIC and did not have clinical evidence of hemorrhage.

Of 3 dogs with type-II responses, 1 had a normal Hct and was not receiving immunosuppressive drugs 21 months after treatment, 1 died of mesenteric torsion 32 days after treatment, and 1 attained a Hct of 29%, which was maintained for 3 months after treatment. This dog ultimately died of acute hepatitis 4 months after hIVIG treatment. At the time of death, the dog was being treated with prednisone and had recently been switched from cyclophosphamide to azathioprine in an attempt to bring Hct into the reference range. Two dogs that had type-I responses died 10 and 42 days after hIVIG treatment because of pulmonary thromboembolism and relapse of anemia, respectively. Two of the 3 dogs that did not have any response after hIVIG treatment died of complications of IMHA within 4 to 7 days after treatment. The other dog that did not respond to hIVIG treatment survived and later responded to treatment with prednisone and azathioprine. This dog ultimately went into full remission and did not require drugs for long-term maintenance treatment. Of 2 dogs that received blood transfusions, 1 had a Hct of 24% after a transfusion 3 days after hIVIG treatment. The Hct continued to increase to 30% without additional treatment, but the dog died suddenly of pulmonary thromboembolism 22 days after hIVIG treatment. In the other dog, which received a transfusion 7 days after hIVIG treatment, Hct varied from 25 to 28% during the 2 weeks after the transfusion. The Hct was normal after addition of azathioprine to the treatment regimen. This dog was alive 12 months after hIVIG treatment. Overall, 7 dogs died. A diagnosis of DIC was made in 2 dogs, and thromboembolism was identified in 5.

Discussion

Immune-mediated hemolytic anemia in dogs is characterized by antibody- and complement-mediated RBC destruction that can result in severe, life-threatening anemia.[1,7,8] Anemia is usually regenerative but can be nonregenerative in some cases.[9-11] Concurrent thrombocytopenia is reported in 60% of cases.[9] Mortality rates reportedly range from 26 to 80%, even with aggressive immunosuppressive treatment and supportive care.[9,12-14] Death may be a result of severe anemia or other complications, such as pulmonary thromboembolism, sepsis, and DIC.[12,15,16] Some breeds, such as Cocker Spaniels, have been reported to have a particularly high mortality rate.[13]

Conventional treatment for IMHA in dogs consists of administration of high doses of prednisone, alone or in combination with a cytotoxic drug such as cyclophosphamide or azathioprine.[1,8] Other treatments that have been reported to be effective in uncontrolled studies[17,18,c] of a small number of dogs include splenectomy and administration of danazol or cyclosporine. A recent report[5] described use of hIVIG in the treatment of 5 dogs with nonregenerative immune-mediated anemia.

Administration of hIVIG at dosages ranging from 400 to 2,000 mg/kg (180 to 900 mg/lb), IV, has been shown to be effective in the treatment of many immune-mediated hematologic diseases in human beings,[19-21] including immune-mediated thrombocytopenia, autoimmune hemolytic anemia, autoimmune neutropenia, and pure RBC aplasia.[3,4,22-24] Administration of hIVIG is now the treatment of choice for children with acute thrombocytopenic purpura.[22] In a study[3] of 73 people with autoimmune anemia, 40% had a type-I response to treatment with hIVIG and 15% had a type-II response. The association of response criteria to long-term outcome was not evaluated. These percentages are similar to those in this study; however, in the human study, most patients received multiple (2 to 7) IV infusions of hIVIG at dosages ranging from 400 to 1,000 mg/kg/d (180 to 450 mg/lb/d).

The mechanism of action of hIVIG in the treatment of immune-mediated diseases is unknown, although there is evidence to support a number of hypotheses. Blockade of Fc receptors on mononuclear phagocytic cells has been proposed as the most likely mechanism for the rapid, early response to hIVIG treatment.[23,25,26] Binding of hIVIG to Fc receptors has been shown to decrease phagocytic activity of human and canine mononuclear cells in vitro.[27] Infusion of hIVIG in patients with idiopathic thrombocytopenia resulted in prolonged clearance of autologous antibody-coated RBC.[23] Other evidence for the role of Fc-receptor blockade is the finding that antibodies directed against the Fc receptor have similar effects to those of hIVIG in patients with idiopathic thrombocytopenic purpura.[28] However, because the half-life of hIVIG in people is only 18 to 32 days,[20] direct Fc-receptor blockade does not explain the long duration of remission observed in some patients after hIVIG infusion. It is possible that binding of hIVIG to Fc receptors has other immunomodulatory effects, such as changes in synthesis or release of cytokines from mononuclear cells.[23,29]

Other researchers have suggested that hIVIG acts to decrease serum concentrations of autoantibodies or to decrease autoantibody production by B cells. The $F(ab')_2$ fragments of hIVIG inhibit the binding of autoantibodies to autoantigens in vitro and neutralize the functional activity of autoantibodies in a dose-dependent manner.[30,31] Autoantibodies are specifically retained on affinity chromatography columns of sepharose-bound hIVIG $F(ab')_2$ fragments.[31] These effects are thought to be a result of anti-idiotypic anti-

bodies in the hIVIG. Anti-idiotypic antibodies may also decrease autoantibody production by modulating the function of the idiotype–anti-idiotype network.[19,30]

Other mechanisms that have been hypothesized to account for effects of hIVIG include functional modulation of T lymphocytes,[30,32] decreased natural killer cell activity,[33] blockade of complement-mediated cell damage,[34,35] and modulation of the release and function of proinflammatory cytokines.[30]

On the basis of criteria established prior to the study, 5 dogs had clinically meaningful improvement (type-I or -II responses) after hIVIG treatment. Seven dogs were still alive 1 month after hIVIG treatment, but only 3 were alive 12 months after treatment. Of these 3, only 1 had had a type-I or -II response. Because this was an uncontrolled study and because the clinical course of IMHA is unpredictable, it is not certain that observed responses were the direct result of hIVIG treatment rather than a delayed response to immunosuppressive drug treatment or a spontaneous improvement. However, similar changes over time in reticulocyte count and Hct in all dogs that responded and the significant linear increase in Hct during the 28 days after treatment suggest that there may have been a cause-and-effect relationship. Although reticulocyte count did increase in most dogs after treatment, there was not a significant linear trend over time. This was probably attributable, in part, to variation between dogs in time between treatment and peak reticulocyte count. Also, as Hct increased, reticulocyte count decreased, which is a normal physiologic response.

The observation that hIVIG treatment may be effective in the treatment of some dogs and people with IMHA provides some insight into the mechanism of action of hIVIG. It seems unlikely that idiotypic antibodies play an important part in response to treatment, because administering a protein derived from another species was of benefit in dogs. Thus, mechanisms such as Fc-receptor blockade or direct neutralization of an infective agent to which dogs and human beings are exposed seem more plausible.

Thrombocytopenia developed some time after hIVIG treatment in 6 dogs in this study. Thrombocytopenia is common in dogs with IMHA[9] and was detected prior to treatment in 3 of these dogs. In our experience, mild transient thrombocytopenia has also been observed in healthy dogs after hIVIG administration. Whether thrombocytopenia that developed after hIVIG treatment in this study was a result of IMHA, DIC, or hIVIG treatment is unknown. In 2 dogs, DIC was strongly suspected on the basis of clinicopathologic data and necropsy findings.

Most dogs that died in this study did not die of anemia, but of complications such as thromboembolic disease, DIC, and mesenteric torsion. Evidence of thromboembolism was detected at necropsy in 5 of 7 dogs that died, and thromboembolism has been reported as a common complication of IMHA in dogs. In 1 study,[15] 32% of dogs treated for IMHA had evidence of pulmonary thromboembolism at necropsy. Risk factors for thromboembolism in that study included hyperbilirubinemia, a negative Coombs' test result, and presence of an indwelling catheter. Pathogenesis of thromboembolism in IMHA is not understood well. Factors hypothesized to play a role include endothelial injury (possibly secondary to immune complex deposition), alterations in blood flow, and an imbalance in endogenous pro- and anticoagulant factors.[15] Whether the high rate of thromboembolism in this study was related to hIVIG treatment or was simply a result of underlying primary disease needs further study.

Immune globulin of canine origin is not commercially available. Antigenicity of hIVIG in dogs is unknown. Administration of other blood-derived or recombinant human proteins to dogs results in clinically important antibody responses after 3 to 4 weeks of administration.[36,37] In a study at Purdue University, we found that the half-life of hIVIG in healthy dogs is 7 to 9 days, which is much shorter than in people.[20] It is possible that an antibody response to hIVIG may result in a short half-life of hIVIG in dogs, and this could explain the lack of long-term responses to hIVIG. The safety of administering multiple doses of hIVIG to dogs has yet to be established. Administration of 2 doses of hIVIG several months apart to dogs with immune-mediated anemia did not result in any apparent adverse effects.[5]

Overall survival rate for dogs in this study was 70% 1 month after treatment and 30% 12 months after treatment. In a review of 23 dogs with IMHA treated at our hospital between 1988 and 1994 that did not receive hIVIG treatment, we found that 10 (43%) were alive 1 month after diagnosis and 8 (36%) were alive 1 year after diagnosis. This suggests that hIVIG treatment did not improve the long-term survival rate in dogs with IMHA. However, dogs in this study had failed to respond to conventional immunosuppressive treatment, and in 2 instances, cause of death did not appear to be directly related to IMHA. Human intravenous immune globulin may be most useful for short-term stabilization of dogs with IMHA.

Two retrospective studies[13,d] have reported use of hIVIG in the treatment of dogs with IMHA. The authors of 1 study[d] believed that hIVIG treatment was of some benefit, but the authors of another study[13] did not report any observable evidence of success with hIVIG treatment. A large, randomized, controlled study is necessary to determine long-term benefits and consequences of hIVIG treatment.

[a]Gammar IV, Armour Pharmaceutical Inc, Collegeville, Pa.
[b]Graphpad Instat, Graphpad Software Inc, San Diego, Calif.
[c]Cook AK, Bertoy EH, Gregory CR, et al. Effect of oral cyclosporine in dogs with refractory immune-mediated anemia or thrombocytopenia (abstr), in *Proceedings*. 12th ACVIM Forum 1994;1001.
[d]Kellerman DL, Bruyette D. Intravenous immunoglobulin in canine immune-mediated hemolytic anemia (abstr), in *Proceedings*. 14th Vet Cancer Soc Annu Conf 1994;10–11.

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