Intravenous Human Immunoglobulin for the Treatment of Immune-Mediated Hemolytic Anemia in 13 Dogs

Dana L. Kellerman and David S. Bruyette

Intravenous immunoglobulin (IVGG) was administered to 13 of 37 dogs with immune-mediated hemolytic anemia. All dogs received concurrent prednisone therapy, 14 dogs also received cyclophosphamide; and a single dog each received cyclosporine, azathioprine, and danazol. Dogs that responded to prednisone therapy without IVGG generally did so within 7 days (mean ± standard deviation = 5.6 ± 2.9 days). Intravenous immunoglobulin was administered after 10.4 ± 6.6 days of prednisone therapy as an intravenous infusion of 0.5 g/kg (range 0.25 to 0.73 g/kg). Eleven dogs received a single treatment, 2 dogs each received 2 treatments. No relevant adverse effects were noted. Eleven dogs had an increase in PCV of at least 4% 2.2 ± 1.5 days after IVGG infusion. In 10 of these dogs, the PCV continued to increase until the time of hospital discharge. One responder died 1 hour after the increase in PCV, 1 dog was euthanized within 24 hours of IVGG administration, and 1 dog had no response over a period of 13 days. Results of this study suggest that IVGG therapy may be of value in dogs with immune-mediated hemolytic anemia that do not respond within 7 days of appropriate corticosteroid therapy.

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Immune-mediated hemolytic anemia (IMHA) is one of the most common immunohematologic diseases and the most common cause of hemolysis in the dog.[1–3] Standard therapy for IMHA includes immunosuppressive doses of corticosteroids, with or without cytotoxic drugs.[1–5] Mortality has been reported to range from 29% to 64%.[1,3,6]

The efficacy of human intravenous immunoglobulin (IVGG) therapy for humans with immune-mediated thrombocytopenia is well established,[7–13] and its use in humans with IMHA has been reported.[14] Human IVGG is reported to be beneficial in 33% to 40% of patients with IMHA.[5,14] The mechanisms of action of IVGG are incompletely defined and may vary with the pathogenesis of the particular disease treated. Proposed mechanisms include Fc receptor blockade, modification of complement activation, decreases in immunoglobulin production, and immune modulation by anti-idiotype antibodies.[15–20] Sustained response to IVGG administration may occur, in part because Fc receptor blockade can last for up to 30 days.[5] Intravenous immunoglobulin may also modify the course of immune-mediated disease by poorly understood mechanisms and can induce long-term remissions.[5,11] Human IVGG has been demonstrated to bind to lymphocytes and monocytes of dogs by an Fc-mediated mechanism.[21] Human IVGG was useful in the treatment of 5 dogs with nonregenerative anemia suspected to be immune mediated in origin.[22] The purpose of this study was to report our experience with human IVGG in dogs with idiopathic IMHA.

Materials and Methods

The records of all dogs with idiopathic IMHA at the Kansas State University Veterinary Medical Teaching Hospital (KSUVMTH) between January 1990 and December 1995 were reviewed. Diagnosis of IMHA was based on the presence of anemia (PCV <35), evidence of hemolysis (spherocytosis, hemoglobinemia, hemoglobinuria, icterus, bilirubinuria), and evidence for erythrocyte-associated antibody (positive direct antiglobulin [Coombs'] test, flow cytometry for IgG on erythrocyte membrane, or presence of autoagglutination without dispersion in saline). Dogs with underlying neoplastic or infectious diseases were excluded. Thirty-seven dogs with idiopathic IMHA were identified. Intravenous immunoglobulin*† was administered to 13 dogs (rIVGG group). Twenty-four dogs with IMHA that did not receive IVGG (nrIVGG group) were used as a comparison group.

Variables evaluated for all dogs included signalment, presence of autoagglutination, intravascular hemolysis, icterus, number of transfusions received, PCV at admission, lowest PCV during hospitalization (PCV nadir), time to response in PCV (defined as an increase of 4 percentage points in the PCV, not related to a blood transfusion), initial reticulocyte production index (RPI), and other medications administered. An increase of 4 percentage points in PCV was selected as an indicator of clinical response because it predicted a sustained increase in PCV in 36 of 37 dogs. The presence of intravascular hemolysis was assessed by the occurrence of hemoglobinuria and hemoglobinemia. The presence of icterus was assessed by physical examination. Because the records on most cases did not include serum bilirubin concentrations, the degree of hyperbilirubinemia could not be evaluated. Results of a direct Coombs' test were available for 20 dogs, and results of flow cytometric analysis for IgG on erythrocyte membranes were available for 18 dogs. Coombs' tests or flow cytometry were not routinely performed on patients in which autoagglutination was present. For dogs that received IVGG, the time from IVGG administration to increasing PCV was evaluated. Because information on medication administered prior to referral to the KSUVMTH was available for all 37 dogs, duration of therapy and time to response were calculated from the first day of glucocorticoid therapy.

Odds ratios (OR) for parameters with yes/no responses were calculated. A *t*-test for independent samples or a nonparametric equivalent (Mann-Whitney test) was used to analyze continuous data (GraphPAD InStat, Statistics Software Package, GraphPAD Software, version 1.10a). Significance was set at $P < .05$. Data are presented as mean ± standard deviation. Where a nonparametric test was performed, medians are also presented. A Kaplan-Meier survival curve was constructed.

* Purified human immune globulin, 5%.

From the Department of Clinical Sciences, Kansas State University, College of Veterinary Medicine, Manhattan, KS.

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Reprint requests: Pittsburg Veterinary Internal Medicine, 882 Butler Street, Pittsburgh, PA 15223.

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Table 1. Patient Characteristics for Dogs Receiving or Not Receiving Human Intravenous Immunoglobulin

	rIVGG (n = 13)	nrIVGG (n = 24)
Signalment		
Breed		
Cocker Spaniel/Cocker Spaniel crosses	3 (23%)	7 (29%)
Shih Tzu	3 (23%)	4 (17%)
Miniature Schnauzer	3 (23%)	1 (4%)
Poodle/Poodle crosses	1 (8%)	3 (13%)
Other	3 (23%)	9 (38%)
Gender		
Female (intact)	0 (0%)	2 (8%)
Female (spayed)	10 (77%)	16 (67%)
Male (intact)	0 (0%)	2 (8%)
Male (neutered)	3 (23%)	4 (17%)
Age (y)	6.4 ± 2.6	5.8 ± 3.0
Weight (kg)	14.6 ± 13.3	13.7 ± 8.6
Hematologic data		
Initial PCV	13.6 ± 4.1	16.7 ± 6.2
PCV nadir	11.3 ± 1.7	13.2 ± 4.4
Platelet count ($\times 10^3/\mu L$)	206 ± 87	228 ± 209
White cell count ($\times 10^3/\mu L$)	28.8 ± 11.1	30.1 ± 18.6
Initial RPI	0.65 ± 0.55	1.33 ± 1.46
Immunologic assessment, characterization of hemolysis		
Coombs' positive	5/7 (71%)	5/13 (38%)
Flow cytometry positive	5/8 (63%)	8/10 (80%)
Autoagglutination	9/13 (69%)	17/24 (71%)
Intravascular hemolysis	2/13 (15%)	4/24 (17%)
*Icterus**	9/13 (69%)	7/24 (29%)
Spherocytosis	8/13 (62%)	13/24 (54%)
Number of transfusions	1.6 ± 1.0	1.0 ± 1.0

* Significant difference between dogs receiving IVGG & those not receiving IVGG ($P < .05$).

Abbreviations: rIVGG, receiving intravenous immunoglobulin; nrIVGG, not receiving IVGG; RPI, reticulocytic production index.

Results

Thirteen dogs received IVGG; 12 dogs received Gammagard (Gammagard S/D, Baxter Healthcare Corporation, Hyland Division, Glendale, CA), and 1 dog received Gamimune (Gamimune N, 5%, Miles Inc, Pharmaceutical Division, Elkhart, IN). Intravenous immunoglobulin was administered as an IV infusion at a dose of 0.5 g/kg (range, 0.25 to 0.73 g/kg) over 4 hours. A single dose of IVGG was given to 11 dogs, 1 dog received 0.25 g/kg on 2 consecutive days, and 1 dog was readmitted and received a second dose of 0.5 g/kg 5 weeks after the initial dose. Only data from the first hospital admission are included in the analysis for this dog.

Dogs were selected to receive IVGG based on an anticipated need for multiple transfusions, progressive clinical course, or perceived lack of response to corticosteroid therapy. The decision to administer IVGG was made by the primary clinician in each case.

Table 1 illustrates patient characteristics at admission. Dogs that received IVGG were similar to the nrIVGG group for age, gender, breed distribution, and initial hematologic assessment. Dogs that received IVGG were more likely to be icteric than dogs that did not receive IVGG (69% versus 29%, OR = 5.5, $P = .045$).

Table 2 shows medications administered. No differences were detected between groups for prednisone dosage, duration of prednisone therapy prior to admission, or the percentage of dogs receiving other cytotoxic or immunosuppressive medications. No difference was detected for the proportion of dogs in each group that received antibiotics or sodium heparin.

Eighteen of 24 (75%) nrIVGG dogs responded while hospitalized; 6 responders (33%) had received CTX. Fourteen of those 18 dogs (78%) responded within 7 days of instituting prednisone therapy. The duration of prednisone therapy prior to response for all 18 dogs was 5.6 ± 2.9 days. The 6 nrIVGG dogs without increases in PCV during hospitalization received prednisone therapy for 16.0 ± 12.7 days (median, 11 days; range, 8 to 41 days). Of these 6 dogs, 2 died (after 8 and 12 days of prednisone), 2 were discharged and had late responses (after 27 and 54 days of prednisone), and 2 were discharged (after 8 and 10 days of prednisone) and lost to follow-up. Both late responders received concurrent CTX.

Response rate in the IVGG group was 85% (11 of 13). Three responders (27%) also received CTX. Intravenous immunoglobulin was administered after 10.4 ± 6.6 days (range, 3 to 27 days; median, 9.0 days) of prednisone therapy. Nine dogs were given IVGG after more than 7 days of prednisone therapy. Dogs without a PCV response by 7 days of prednisone therapy were more likely to respond during hospitalization if they received IVGG than if they did not (89% versus 40%, OR = 12.0, $P = .039$). Survival was similar between the rIVGG and nrIVGG groups (77% versus 87%, $P > .05$) (Fig 1).

The time to response after IVGG administration was 2.2 ± 1.5 days (range, 0.6 to 6.0 days). In 10 dogs, the increased

Table 2. Drugs Administered to Dogs Receiving and Not Receiving IVGG

Drug	rIVGG (n = 13)	nrIVGG (n = 24)
Prednisone	13/13 (100%)	24/24 (100%)
Prednisone dosage (mg/kg/d)	3.9 ± 0.6	4.2 ± 0.82
Cyclophosphamide (200 mg/m²/w)	6/13 (46%)	8/24 (33%)
Other immunosuppressive drugs*	1/13 (8%)	1/24 (4%)
Antibiotics	5/13 (38%)	14/24 (58%)
Ampicillin/amoxicillin/cephalosporin	4/13 (31%)	7/24 (29%)
tetracycline or oxytetracycline/ doxycycline	2/13 (15%)	5/24 (21%)
Gentocin	2/13 (15%)	0/24 (0%)
Enrofloxacin	1/13 (8%)	3/24 (13%)
Trimethoprim-sulfamethoxazole	0/13 (0%)	2/24 (8%)
Other	0/13 (0%)	2/24 (8%)
Heparin	8/10 (80%)	12/20 (60%)

* Other immunosuppressive agents were cyclosporine and danazol (1 dog in the rIVGG group) and azathioprine (1 dog in the nrIVGG group).

Abbreviations: rIVGG, receiving intravenous immunoglobulin; nrIVGG, not receiving IVGG.

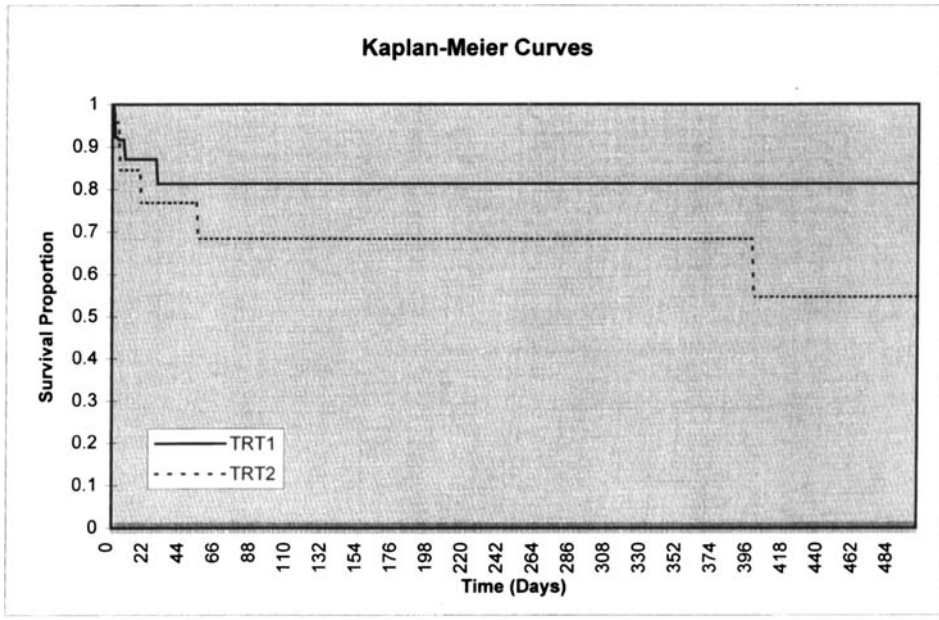

Fig 1. Kaplan-Meier survival curves for dogs with immune-mediated hemolytic anemia treated with and without intravenous human immunoglobulin (IVGG). TRT1; dogs that received IVGG; TRT2; dogs that did not receive IVGG.

PCV was sustained and progressive until hospital discharge (Fig 2). One dog died an hour after the increased PCV, so it could not be determined if the increase would have been sustained. One dog was euthanized within 20 hours of receiving IVGG because the PCV continued to decline. One dog had no response over a period of 13 days and was euthanized. Four of the rIVGG dogs had an RPI <1 at the time of IVGG administration; all of these dogs responded. Seven of the 9 rIVGG dogs with an RPI >1 at the time of IVGG administration responded.

No major adverse effects were directly attributed to IVGG administration. One dog vomited during the IVGG infusion; however, this dog had been vomiting prior to drug administration. One dog died an hour after an increase in PCV that followed IVGG administration. Necropsy revealed severe pulmonary thromboembolism and pulmonary edema. Both pulmonary disorders had been diagnosed prior to IVGG administration. It is possible that the increased PCV in this case could have been due to hemodynamic changes prior to death.

Ten dogs that received IVGG were discharged from the hospital. One dog died of presumptive hepatic failure at 367 days. One dog was euthanized at 53 days following a relapse of IMHA. The other 8 dogs are still alive at a median of 460 days (range, 120 to 1,075 days). Seven dogs are no longer receiving any medication for IMHA. One dog developed a second episode of IMHA when her prednisone dosage was decreased to 0.5 mg/kg every other day 3 months after hospital discharge. The anemia responded to an increased dosage of prednisone (2 mg/kg PO bid) and azathioprine (2.2 mg/kg PO sid).

Discussion

The rapid increase in PCV after IVGG administration (Fig 2) suggests that IVGG is beneficial in some dogs with IMHA. In people, Fc receptor blockade caused by IVGG occurs almost immediately, and immunomodulatory effects may be seen within 3 days.[5] In children with immune-mediated thrombocytopenia, an increase in platelet count occurs within 72 hours of IVGG administration, but in the majority (79%), it occurs within 24 hours.[7,11] The rapid response seen in the dogs in this study is consistent with this time frame.

The 85% response rate seen in our study is higher than the 33% to 40% rate reported in human patients with IMHA.[5,8,14] In human beings, lower initial hemoglobin concentration was a predictor of good response to IVGG, with 73% of patients with hemoglobin <7.0 g/dL having a response.[14] All dogs in the rIVGG group had a hemoglobin concentration of ≤ 7.0 g/dL.

Intravenous immunoglobulin was effective in dogs with both regenerative and nonregenerative anemia. The rapid response in dogs with nonregenerative IMHA suggests that an immune-mediated mechanism for the apparent arrest of erythroid precursors was present in these cases. Intravenous immunoglobulin has been reported to be beneficial in 5 dogs with nonregenerative anemia.[22]

A delayed response to glucocorticoids in the IVGG-treated group is unlikely. Dogs that responded to prednisone (without IVGG) generally did so within a relatively short time frame. Although the nrIVGG dogs without an early response were observed for as long a period of time as the dogs that received IVGG, response rate in this group remained low. Response rates for the dogs receiving IVGG were excellent, even though most of these dogs did not receive IVGG until they demonstrated a lack of response to 7 days of prednisone. Cyclophosphamide administration is unlikely to have been responsible for the responses in the rIVGG group because only 3 of the 11 rIVGG responders received CTX.

We were unable to assess the use of IVGG in dogs with severe disease (intravascular hemolysis, high transfusion re-

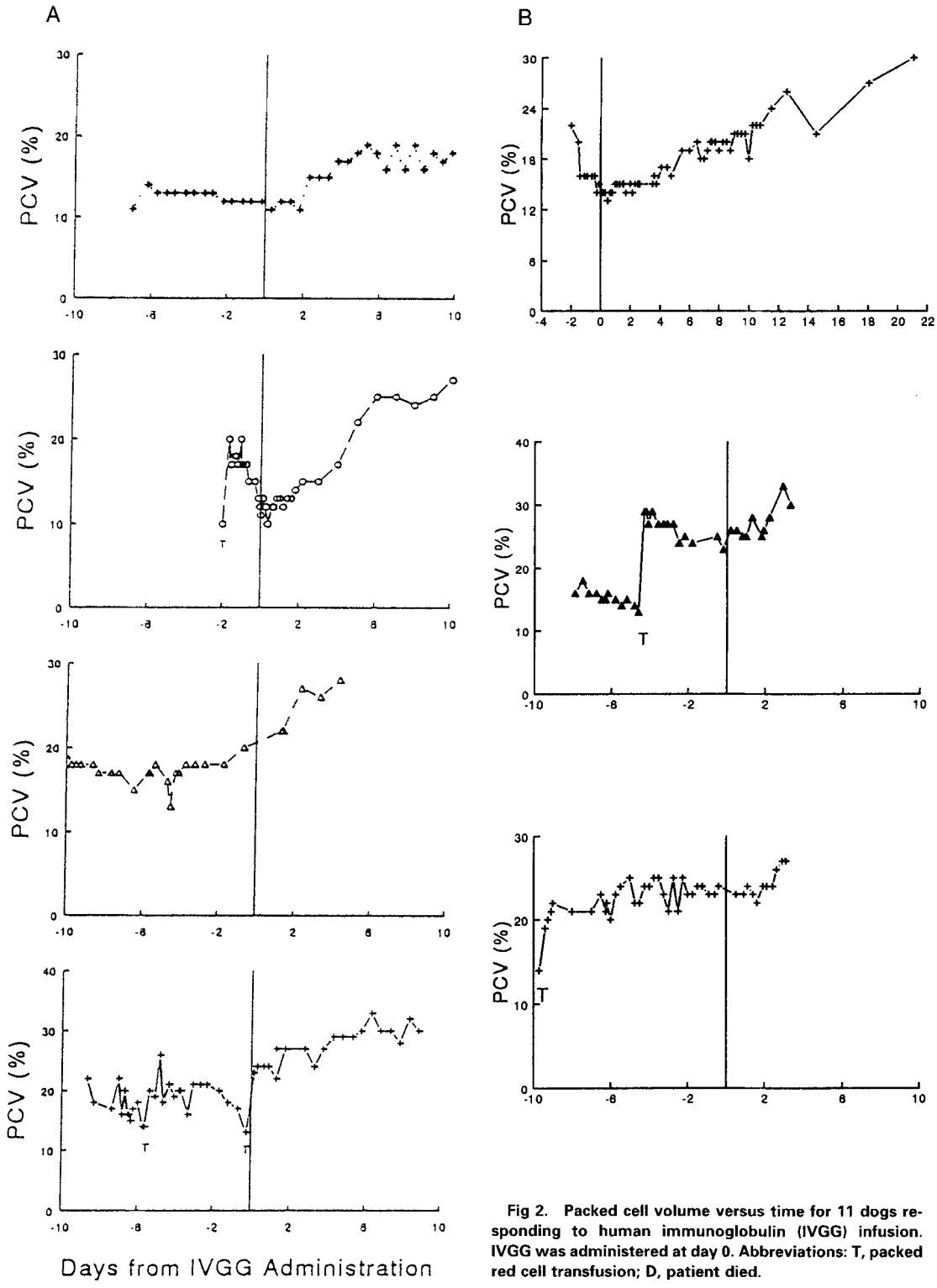

Fig 2. Packed cell volume versus time for 11 dogs responding to human immunoglobulin (IVGG) infusion. IVGG was administered at day 0. Abbreviations: T, packed red cell transfusion; D, patient died.

quirement) prior to 7 days of corticosteroid therapy. Although it is possible that IVGG may be of value in these dogs, we were not able to define prognostic factors to distinguish these dogs from dogs likely to respond to prednisone alone. Further studies may help define the population of patients most likely to benefit from IVGG.

Although we saw no adverse effects that could be directly attributable to IVGG administration, IVGG is a human

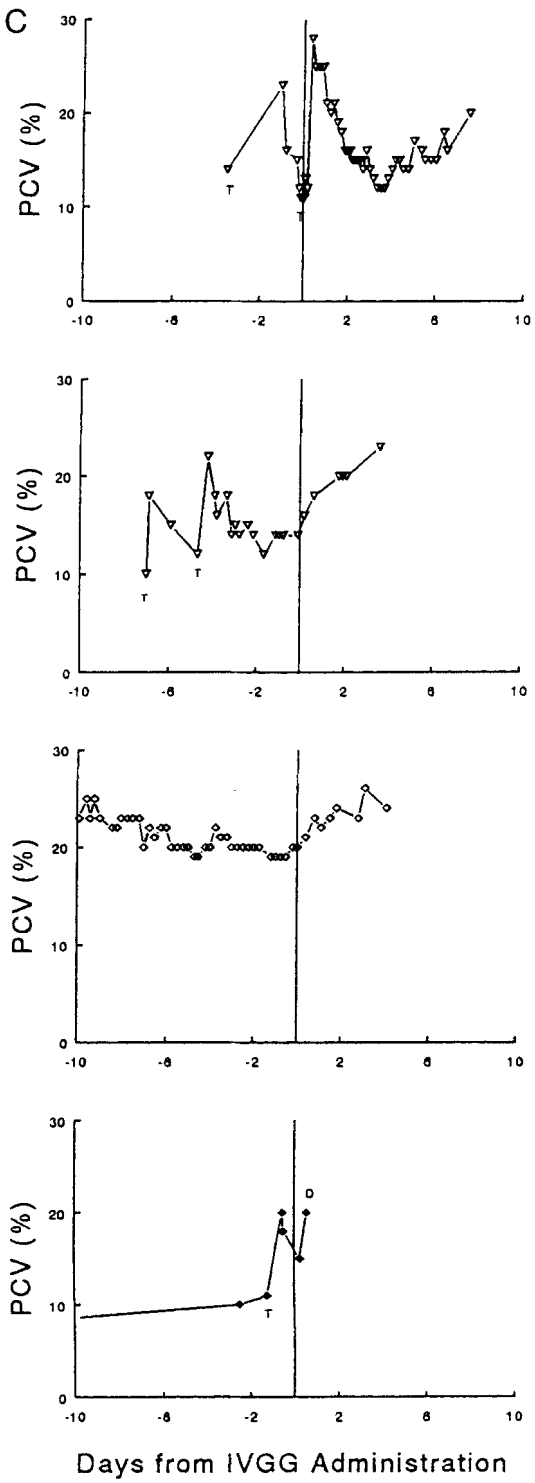

Fig 2. (Cont'd)

plasma product, and the potential for hypersensitivity reactions exists. Dogs may develop antibodies to the human immunoglobulin, so the safety of repeated doses of IVGG is unknown. In humans, severe adverse effects of IVGG have a prevalence of less than 4% and include aseptic meningitis, renal failure, and hemolytic anemia.[23] Intravenous immunoglobulin therapy appears to be of benefit in dogs with IMHA that do not develop an increasing PCV within 7 days of initiation of corticosteroid therapy. This population is unlikely to respond to continued steroid therapy alone, and response to CTX or other cytotoxic drugs may take 1 to 3 additional weeks. Although the cost of IVGG may be substantial, this should be weighed against the potential benefits of rapidly increasing PCV, shortened hospital stay, and potential decrease in transfusion requirements.

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