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Full Length Research Paper

Benign monoclonal gammopathy in a dog exhibiting a low antibody titer to *EHRLICHIA CANIS*

Stephanie Ng[1], Sophia Lim[1], Allison Daub[2], Surinder S. Saini[1] and Azad K. Kaushik[1,3]*

[1]Department of Pathobiology, University of Guelph, Guelph, Ontario, Canada N1G 2W1, Canada.
[2]Campus Estates Animal Hospital, Guelph, Ontario, Canada N1G 3A2, Canada.
[3]Department of Molecular and Cell Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1, Canada.

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The occurrence of a remarkable benign γ-monoclonal gammopathy in a 2.5-year-old intact male bichon frise dog, secondary to acute *EHRLICHIA CANIS* infection, with no travel history outside of Ontario. Exceptionally high levels of serum IgG (104.0 g/ L) as a result of benign monoclonal gammopathy were noted during the disease state that returned to normal subsequent to recovery. Treatment with doxycyline and prednisone resulted in complete recovery and disappearance of the monoclonal gammopathy. In conclusion, such a recovery from monoclonal gammopathy due to infection is indeed promising since very high serum IgG concentrations can be brought back to physiological levels by therapeutic intervention.

Key words: Dog, benign monoclonal gammopathy, *Ehrlichia canis.*

INTRODUCTION

Canine ehrlichiosis, a tick-borne rickettsial disease caused by *Ehrlichia canis* (Skotarczak, 2003; Sambri et al., 2004; Foglia et al., 2006; Stich et al., 2008), is thought to be of rare occurrence in Canada. Though majority of the cases occur in the southwestern United States (Cohn, 2003), but seropositive dogs have been identified throughout the United States (Woody and Hoskins, 1991). A search of the Ontario Veterinary College-Veterinary Teaching Hospital's records revealed 14 confirmed or tentatively diagnosed cases of canine ehrlichiosis (12 *E. canis*; 1 *Ehrlichia risticii*; 1 *Ehrlichia platys*) from January 1988 to December 1999, without taking into consideration the travel history of animals. Given the trend for geographic spread of canine ehrlichiosis in North America, it is important to consider the possibility of *E. canis* infection as a differential diagnosis in dogs suspected for monoclonal gammopathies in Canada.

A wide variety of clinical and hematologic abnormalities, such as hypergammaglobulinemia, lymphadenopathy, pancytopenia, Coombs' positive anemia, polyarthritis, and plasma cell infiltration into tissues, have been ob-served in canine ehrlichiosis (Breitschwerdt et al., 1987; Woody and Hoskins, 1991; Varela et al., 1997). These characteristics suggest the involvement of immune-mediated mechanisms in the pathophysiology of canine ehrlichiosis, leading to the development of monoclonal gammopathy. We present a case of a significant γ-mono-clonal gammopathy in a 2.5-year-old, intact male bichon frise dog with a positive antibody titer to *E. canis* without any history of travel outside southern Ontario. Given animal transportation needs globally, the potential role of canine sentinels, reservoirs and models of tick-borne zoonoses (Stich et al., 2008) deserves consideration.

MATERIALS AND METHODS

Subject

A dog was presented for vomiting, depression, lethargy, occasional lameness and inappetance of 3 days duration. Physical examination revealed mild pyrexia (39.7°C), pale mucous membranes, pain in the left forelimb, and an enlarged spleen. Abdominal radiographs confirmed splenomegaly.

*Corresponding author. E-mail: akaushik@uoguelph.ca. Fax: (519) 837 1802.

Clinical investigations, differential diagnosis and treatment

Urinalysis showed proteinuria (1 g/L), bilirubinuria, hematuria, and hemoglobinuria with a urine specific gravity of 1.036. A complete blood cell count (CBC) revealed a normocytic, normochromic, moderate anemia (hematocrit 0.26 L/L; normal, 0.38 to 0.57 L/L) with mild polychromasia and anisocytosis, thrombocytopenia (48 × 10^9/ L; normal, 140 to 400 × 10^9/l), decreased mean platelet volume (5.2 fl; normal, 5.4 to 7.8 fl) and monocytosis (1.63 × 10^9/L; normal, 0.1 to 1.4 × 10^9/L). Serum biochemistry (Table 1) abnormalities included elevated total protein (96 g/L; normal, 55 to 76 g/l), hypoalbuminemia (17 g/l; normal, 28 to 38 g/l), marked hyperglobulinemia (79 g/l; normal, 24 to 41 g/l), decreased serum creatinine (38 ∝mol/L; normal, 63 to 124 ∝mol/l) and elevated creatine kinase (1532 U/l; normal, 52 to 200 U/l). A rapid slide agglutination test was negative for autoagglutination indicating absence of IgM autoantibody associated with red blood cells. The dog was treated with 0.3 ml of dexamethasone, subcutaneously (SC) and sent home on 5 mg of prednisone, per os (PO), q12h for 3 d. A CBC performed the next day was similar, except that a mild lymphopenia (0.9 × 10^9/L; normal, 1.0 to 4.8 × 10^9/L) was evident and platelets had returned to low normal numbers (200 × 10^9/L; normal, 200 to 500 × 10^9/L), presumably in response to dexamethasone treatment (Table 1). The systemic autoimmune disease, multiple myeloma, and ehrlichiosis were considered for the purpose of differential diagnosis.

RESULTS

Exceptionally high levels of serum IgG in the absence of myeloma

Four days following presentation, full body radiographs did not show osteolytic lesions indicative of myeloma. On examination of bone marrow biopsy, erythroid hyperplasia presumably as part of a regenerative anemia, granulocytic hypoplasia and increased plasma cells, without evidence of malignancy were diagnosed. The activated clotting time (ACT) was significantly prolonged at 6 min, and the buccal mucosal bleeding time was also prolonged. Serum electrophoresis demonstrated a dramatic increase in β-2 globulins (42 g/L; reference range, 3 to 7 g/L), and immunoglobulin quantification by radial immunodiffusion revealed exceptionally high levels of serum immuno-globulin G (IgG) (104.0 g/L; normal 10.0 to 20.0 g/L) (Table 2). The dog was placed on doxycycline, 5 mg/ kg body weight, PO q12h as a precaution, and prednisone was increased to 10 mg, PO, q12h for 14 days.

Benign monoclonal gammopathy revealed by serum and urine immunoelectrophoresis

Two weeks after the onset of clinical signs, the antibody titer (1:80) to *E. canis* was observed. Paired serum testing demonstrating a rising antibody titer is necessary to diagnose active *E. canis* infection. However, due to owner constraints, acute and convalescent titers were not determined. Immunoelectrophoresis (IEP) performed on serum and urine revealed an elongated dense precipitin arc for IgG (Figure 1). The high IgG levels suggested either monoclonal or oligoclonal gammopathy, distinct from polyclonal since the IgM and IgA profiles were normal. The distinction between monoclonal and oligoclonal gammopathy is not made due to the limitations of the IEP technique. Whether a gammopathy is truly of monoclonal or oligoclonal origin can only be determined by clonotypic analysis of variable-diversity-joining (VDJ) rearrangements from B-lymphocytes. In a clinical setting, however, IgG subclass typing may help exclude monoclonal gammopathy. Clinically, the term monoclonal gammopathy is preferred in order to differentiate it from polyclonal gammopathy based on qualitative IEP evaluation.

In contrast to the persistently low hematocrit (26%) and hyperproteinemia, an improvement in the dog's attitude and activity level was apparent following treatment with doxycycline. Three weeks following onset of clinical signs, IEP on urine was positive for γ-immunoglobulin isotype reflecting renal glomerular injury. Glomerulonephritis and IgG proteinuria have been documented in canine ehrlichiosis (Breitschwerdt et al., 1987). Four weeks after the onset of symptoms, the hematocrit increased to 36% and serum total protein levels were within the normal range. Doxycycline was discontinued, but prednisone was administered for an additional 7 days, then reduced to zero over a 2 week period. Eight months following onset of clinical signs, the hematocrit was within the normal range (51%). Serum immunoglobulin determinations 10 months following the initial quantification, demonstrated that IgG levels had returned to within the normal range. Serum and urine IEP performed at the same time confirmed disappearance of the IEP pattern (Figure 1).

DISCUSSION

Canine ehrlichiosis is a common disease in southwestern United States but occurs rarely in Canada (Anderson and Lust, 1999; Cohn, 2003). In this case, the antibody titer to *E. canis*, though relatively low (1:80), was indicative of *E. canis* exposure. It should be noted that the antibody titer to *E. canis* might have been artificially low since prednisone treatment lowers antibody production non-specifically (Drouet et al., 1999a; Waner et al., 2001). This particular dog showed symptoms of canine ehrlichiosis without traveling outside Ontario, therefore, the possibility of existence of *E. canis* tick vector (*Rhipicephalus sanguineus*) or alternate vector in Ontario cannot be excluded. A significant monoclonal gammopathy was observed during disease state in this dog. Several criteria may be used for differentiating monoclonal gammopathy caused by *E. canis* from multiple myeloma: (1) Presence or absence of osteolytic lesions; (2) Positive *E. canis* titer; (3) Age (mean age for multiple myeloma is 8.5 years); 4) Bence Jones proteins in urine (higher occurrence in multiple myeloma) (Varela

Table 1. Hematological and biochemical profile of the Bichon frise dog.

Test	Results		Reference values	Units
	07/23/99	07/24/99		
Hematology				
WBC	12.5	15.6	5.5 - 16.4	10^9/L
RBC	3.7	3.9	5.6 - 8.7	10^{12}/L
Hb	84	91	132 - 193	g/L
HCT*	0.26	0.29	0.38 - 0.57	L/L
MCV	69	73	63 - 77	fL
MCH	23	23	21 - 25	pg
MCHC	330	317	315 - 360	g/L
Reticulocytes		0.8	0 - 1.5	%
RDW	15.0		11.5 - 15.0	%
Platelets	48	200	140 - 400	10^9/L
MPV	5.2		5.4 - 7.8	fL
T.S. Protein	87		55 - 75	g/L
Neutrophils		11.4	3.0 - 11.5	10^9/L
Seg. neutrophils	8.38		3.2 - 11.0	10^9/L
Band neutrophil		0.2	0.0 - 0.3	10^9/L
Lymphocytes	2.00	0.9	0.5 - 3.4	10^9/L
Monocytes	1.63	2.8	0.1 - 1.4	10^9/L
Eosinophils	0.50	0.3	0.0 - 1.2	10^9/L
Basophils		0	0.0 - 0.2	10^9/L
Nucleated RBC		0	0	/100WBC
Polychromasia	0-2	0 - 1		/100×
Anisocytosis	2+	mild		
Rouleaux	2+	mild		
Biochemistry				
Calcium	2.17		2.3 - 2.9	mmol/L
Phosphorus	1.16		0.95 - 1.94	mmol/L
Magnesium	0.9		0.7 - 1.6	mmol/L
Sodium	142		143 - 158	mmol/L
Potassium	4.4		3.8 - 5.4	mmol/L
Chloride	114		104 - 122	mmol/L
Carbon dioxide	17		13 - 24	mmol/L
Anion Gap	15		16 - 32	mmol/L
Total protein**	96	103	55 - 76	g/L
Albumin	17	19	28 - 38	g/L
Globulin	79		24 - 41	g/L
A:G ratio	0.22	0.2	0.67 - 1.40	
Urea	4.5		3.0 - 11.3	mmol/L
Creatinine	38		63 - 124	umol/L
Glucose	5.7		3.7 - 6.1	mmol/L
Cholesterol	3.13		3.0 - 10.0	mmol/L
Total bilirubin	3		0 - 4	umol/L
Conj. bilirubin	1		0 - 1	umol/L
Free bilirubin	2		0 - 3	umol/L
Alk-phos	80		17 - 86	U/L
S-Alk phos	0		0 - 35	U/L
Gamma-GT	3		0 - 6	U/L
ALT	65		14 - 91	U/L

Table 1. Contd.

CK	**1532**	52- 200	U/L
Amylase	1031	300- 1500	U/L
Lipase	79	0 - 600	U/L
N:K Ratio	32	24 - 45	
Calc. Osmolarity	283		mmol/L

*HCT 08/03/99 0.26L/L; 08/16/99 0.36L/L; 03/17/00 0.51L/L; **TP 08/03/00 90g/L; 03/17/00 68g/L.

Table 2. Immunological findings in a 2.5-year-old a bichon frise dog with benign gammopathy.

Serum electrophoresis in disease state (07/26/99)		
Test	**Result(g/L)**	**Reference values (g/L)**
Total Protein	105	55 - 76
A:G	0.2	0.67 - 1.40
Albumin	17	26 - 38
Globulin	88	24 - 34
Alpha 1	9	5 - 8
Alpha 2	13	5 - 8
Beta 1	9	5 - 11
Beta 2	42	3 - 7
Gamma Globulins	15	3 - 8

Immunoglobulin levels (g/L) in disease state and post-recovery			
Ig class	**Disease state (07/28/99)**	**Post-recovery (05/17/00)**	**Reference values**
IgM	2.2	2.2	1.0 - 2.0
IgG	104.0	17.9	10.0 - 20.0
IgA	0.14	0.2	0.4 - 1.6

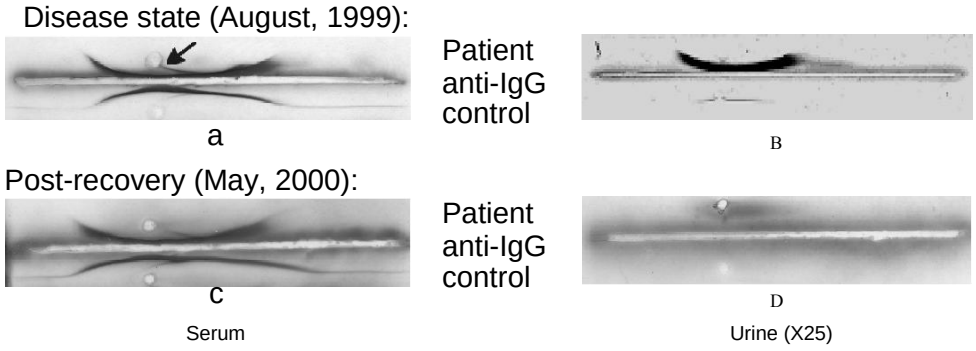

Figure 1. Disease state (a, b) and post-recovery (c, d) immunoelectrophoretic profile for IgG (γ) in serum (a, c) and urine (b, d) as compared to normal control. Note the elongated and dense γ-arc in the disease state serum (a) and urine (b) and its disappearance in urine (d) post-recovery.

et al., 1997). In the presented case, no osteolytic lesions were found and a bone marrow biopsy showed granulocytic hypoplasia and an increase presenc of plasma cells, characteristic of *E. canis* infection (Breitschwerdt et al., 1987; Andreone et al., 1998; Anderson and Lust, 1999). The presence of Bence Jones proteins in urine was not examined by using anti-light chain specific antibodies in IEP. A positive *E. canis* titer in a non-endemic area associated with clinical signs and the dog's young age (2.5 years) were considered to be most compatible with benign monoclonal gammopathy, likely secondary to *E. canis* infection (Waner et al., 2001). The

suspicion of *E. canis* infection was further confirmed by the quick response to doxycycline therapy resulting in complete recovery.

A negative slide agglutination test helped to rule out IgM-mediated immune-mediated hemolytic anemia (IMHA) secondary to *E. canis* infection. However, the presence of pale mucous membranes, splenomegaly, bilirubinuria, hematuria, and persistent anemia with increased IgG levels were suggestive of IgG-mediated red blood cell (RBC) destruction. A direct or indirect Coombs' test would have helped to demonstrate the occurrence of IgG autoantibody to RBCs with immunopathogenesis associated with *E. canis* infection. Mononuclear cells, principally monocytes, are infected with *E. canis*. The newly emerged cryptic and/ or neo-epitopes on monocytes as a result of *E. canis* invasion may be shared with normal RBC surface antigens, and thus might be responsible for triggering IgG-mediated IMHA.

The dog experienced pain and lameness, characteristic of polyarthritis described in *E. canis* infected dogs and may be due to immune complex deposition in joints (Breitschwerdt et al., 1987; Andreone et al., 1998; Anderson and Lust, 1999). The possibility of additional underlying immune-mediated factors responsible for these symptoms is not excluded. Immunological perturbbation caused by monoclonal gammopathy, may at least in part, contribute to these symptoms.

Some unique characteristics not reported earlier in dogs suffering from ehrlichiosis were noted: increased ACT, low mean platelet volume (MPV), increased serum creatine kinase (CK), and decreased serum creatinine. Coagulation tests performed on *E. canis* infected dogs, with or without thrombocytopenia, typically reveal a prolonged buccal mucosal bleeding time, while the ACT and other screening tests remain normal. Hemorrhagic tendencies have been attributed to thrombocytopenia and platelet function defects (Woody and Hoskins, 1991). Monoclonal proteins are thought to interfere with normal platelet function by binding to platelets and the vascular endothelium (Breitschwerdt et al., 1987; Andreone et al., 1998). Eighty to nine percent of dogs with experimentally induced subclinical *E. canis* infection, experience a significant increase in MPV in response to immune-mediated platelet destruction (Kelly et al., 1985). In the presented case, the decreased MPV can be attributed to platelet fragments as a consequence of destruction. A single injection of dexamethasone led to a remarkable recovery of platelets within 24 h. The increased serum CK levels and concomitant decline in serum creatinine suggest a muscle related disorder.

The low antibody titer to *E. canis*, clinical signs, and a quick response to treatment are compatible with the acute phase of *E. canis* infection. However, the profound increase in total IgG levels seen in this dog is most often experienced in the chronic state. Most IgG produced in this dog likely reflected oligoclonal B-cell activation and proliferation, leading to spontaneous IgG secretion, not necessarily directed against *E. canis* antigens (Anderson and Lust, 1999). It appears that the *E. canis* organism may possess B-cell superantigen activity capable of activating a significant population of B-cells upon infection. Whether *E. canis* also has superantigen activity for T cells is unknown and needs to be investigated. Since interleukin–6 (IL-6) and IL-1β are known to be important in conversion of monoclonal gammopathy of undetermined significance (MGUS) to multiple myeloma in people (Rinkardt, et al., 1999), it would be useful to monitor IL-6 levels in dogs afflicted with canine ehrlichiosis. Other potential infectious causes of monoclonal gammopathy include canine leishmaniasis, and feline infectious peritonitis. Benign monoclonal gammopathies in humans are also known to be associated with a few viral hepatitis C (Varela et al., 1997; Andreone et al., 1998), *cytomegalovirus* and *Epstein-Barr viruses* (Drouet et al., 1999b) and *Corynebacterium pyogenes* (Norenberg et al., 1978) infections. Although the majority (63%) of monoclonal gammopathies are associated with lymphoid disorders in people, it would be prudent to consider both acute and chronic bacterial, viral, and rickettsial infections that may result in benign monoclonal gammopathy. The suggested treatment strategy in such conditions, therefore, must be directed at eliminating the infection by using the appropriate antibiotics and if necessary, decreasing the immune complex load through plasmapheresis. The treatment strategy used here that resulted in recovery from monoclonal gammopathy is consistent with a previous study (Heeb et al., 2003).

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