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Anti-histone antibodies in dogs with leishmaniasis and glomerulonephritis

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Abstract

The association between serum anti-histone antibodies and glomerulonephritis was studied in 43 dogs with leishmaniasis (*Leishmania infantum*). Dogs with increased serum creatinine levels and urine protein-creatinine ratio >1 were considered to have glomerulonephritis. Moderately elevated anti-histone antibodies were found in 38.89% (7/18) of infected dogs without glomerulonephritis, whereas 88% of dogs with glomerulonephritis (22/25) showed moderate or strongly elevated anti-histone antibodies. Prevalence of positive anti-histone antibodies reactions and mean serum concentration was significantly higher ($P < 0.001$; $P < 0.0001$) in infected dogs with glomerulonephritis. Correlation between anti-histone antibodies and urine protein-creatinine ratio was significant when groups were analysed together ($P < 0.046$). Positive predictive value for glomerulonephritis of positive anti-histone antibodies was 88%. In conclusion, high anti-histone antibodies are significantly associated with glomerulonephritis. Although other factors must be involved, dogs with moderate or strong positive anti-histone antibodies reactions may have a higher probability to develop glomerular lesions in canine leishmaniasis.

Keywords: Antinuclear antibodies; Histones; Leishmaniasis; Glomerulonephritis; Dog

1. Introduction

The term antinuclear antibodies (ANA) is used in a generic way to describe autoantibodies against different nuclear components such as DNA, RNA, histones and their molecular complexes (Tan, 1982). Antinuclear antibodies contribute to vascular immune complex deposition and subsequent type III hypersensitivity reactions usually manifested clinically by glomerulonephritis, dermatitis, uveitis and arthritis (Monestier et al., 1995; Hahn, 1998). In dogs, ANA are mainly associated with SLE although can also be found in other inflammatory and infectious diseases such as canine leishmaniasis (Kelly et al., 1994; Lucena et al., 1996; Paul et al., 2005). As in canine SLE, glomerulonephritis is often reported in canine leishmaniasis and similarly, its association with glomerular deposition of immune complexes and subsequent renal impairment has been demonstrated (Poli et al., 1991; Zatelli et al., 2003).

In contrast with human SLE patients, dogs with SLE commonly show anti-histone antibodies (AHA) whereas the prevalence of ANA specific for double stranded DNA is controversial (Monestier et al., 1995; Paul et al., 2005). Histones are thought to play a central role in the binding of circulating immune complexes to the glomerular basement membrane (GBM). Immune complexes do not bind to the GBM unless they are strongly cationic, whereas the DNA–anti DNA immune complexes are anionic. Therefore, the cationic histones as well as nucleosomes have been implicated in the deposition process (Teodorescu et al., 2004). Thus, histones seem to be a key antigen in the development of immune-mediated glomerulonephritis and the study of AHA in canine leishmaniasis can be of interest

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to determine their association with the glomerular lesions described in this disease.

The objectives of this prospective study were to determine the prevalence of AHA positive titres in sera from dogs with leishmania infection and assess their correlation with glomerulonephritis to investigate their pathogenic role. Also, a significant association between positive AHA and glomerulonephritis could be clinically relevant to assess the risk of glomerular dysfunction in dogs with leishmaniasis.

2. Materials and methods

2.1. Animals

Dogs were included in the study prospectively. Diagnosis of leishmaniasis was made by the presence of amastigotes in lymph node smears or by a positive PCR in blood samples. In all dogs, a detailed history and a systematic clinical examination was performed, including thoracic radiographs and abdominal ultrasound studies. Peripheral blood samples were collected in EDTA-containing tubes for blood cell count and in lithium heparin for biochemistry analysis. The sera of all dogs were checked for specific antibodies against *Ehrlichia canis, Anaplasma platys,* and *Neorickttesia risticii* by indirect fluorescent antibody test (IFAT; Eurovet Lab.). Urine samples were taken by urinary bladder catheterisation and a complete urinalysis with attention to the urine protein-creatinine (UPC) ratio and microscopic sediment observation was performed. The UPC ratio was calculated as the urine concentration of protein (Lowry method) divided by the urine concentration of creatinine, with both expressed as mg/dl (DiBartola, 2000). In our laboratory normal UPC ratios in dogs are generally <0.5, but a cut-off value of >1 was chosen to make the parameter more specific in the diagnosis of significant glomerular disease. All dogs with UPC >1 had increased serum creatinine levels.

Criteria for exclusion in all groups were a positive titre for any of the infectious diseases tested, age greater than 5 years, presence of clinical signs not considered typical of leishmania infection such as cardiorespiratory and gastrointestinal signs, and evidence of post-renal proteinuria based on urinalysis and microscopic sediment evaluation. All procedures applied in this study were approved by the Committee of Animal Ethics of the University of Cordoba, Spain.

Fifty clinically normal dogs (22 males, 28 females, age range 0.5–5 years) and 43 dogs with natural leishmaniasis (29 males, 14 females, age range 1.5–5 years) entered the study. All dogs came from the south of Spain. The group of normal dogs included blood donors and pet dogs. Dogs with leishmaniasis were distributed in two groups depending on the degree of renal impairment evaluated by serum creatinine levels and UPC ratio. Group I included 18 dogs with clinical signs of leishmaniasis and normal renal function. Group II was composed of 25 dogs with classical signs of leishmania infection and signs of glomerular involvement (UPC ratio >1 and serum creatinine concentration >1.3 mg/dl).

2.2. Determination of anti-histone antibodies

Anti-histone antibodies were determined by an ELISA method using purified bovine histones directly absorbed onto micro-ELISA plates (Monestier et al., 1995). All sera were tested in duplicate. Sera from 50 healthy dogs were used as negative controls. Briefly, micro-ELISA plates (Maxisorb, Nunc) were coated 2 h at 37 °C with purified bovine histones (total histones from calf thymus, type II-S; Sigma–Aldrich) diluted in 0.05 M carbonate buffer, pH 9.5. The optimal amount of protein for coating the wells, as determined by titration, was 2.5 μg/ml. After each step, plates were washed six times with PBS–0.05% Tween 20. Uncoated protein-binding spots were blocked by incubation for 2 h at 37 °C with 1% bovine serum albumin (BSA) in PBS. Serum samples were diluted 1:100 in PBS–0.05% Tween 20, 0.5% BSA and incubated 1 h at 37 °C. Biotin conjugated anti-dog IgG antibodies (Sigma–Aldrich) were diluted 1:1000 in PBS–0.05% Tween 20, 0.5% BSA and the plates were incubated for 1 h at 37 °C. Extravidine-peroxidase conjugate (Sigma–Aldrich) at 1:2000 dilution in PBS–0.05% Tween 20, 0.5% BSA was added and the plates incubated 1 h at 37 °C. Finally, the peroxidase reaction was determined after incubation for 15 min at room temperature with 2,2′-azino-di-3-ethyl-benzothiazoline sulphonic acid (ABTS, Sigma–Aldrich) and hydrogen peroxide 35% (ABTS 0.5 mg ml^{-1} + H_2O_2 1 μl ml^{-1} of 1 M citrate buffer, pH 5). The absorbance of the peroxidase reaction was measured at 405 nm with a microtitre plate reader (ELX 800; Bio-Tek instruments INC).

Optical density (OD) readings after background subtraction were divided by 2 SD above the mean value of the healthy controls to obtain ELISA units (Henriksson et al., 1998). Reactions <1 unit were considered negative. Positive reactions were graded as weak (1–1.5 units); moderate (1.6–2.5 units) and strong (>2.5 units). All statistical calculations were performed using GraphPad prism 5 (GraphPad Software, San Diego, USA).

3. Results

3.1. Clinical and laboratorial findings

On the basis of serum creatinine levels and UPC ratio 18 dogs were allocated to group I and 25 dogs to group II, and their clinical and laboratory findings tabulated separately. All the animals with leishmaniasis were symptomatic but a differentiation could be made between dogs showing multiple signs (weight loss, enlargement of lymph nodes, cutaneous ulcers, dry seborrhoea, onycodistrophy, keratoconjunctivitis…) and those that could be classified as olygosymptomatic (mainly weight loss and lymph node