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Development and evaluation of a flow cytometry microsphere assay to detect anti-histone antibody in dogs

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Abstract

Anti-nuclear antibody (ANA) is one of the diagnostic parameters that support a diagnosis of autoimmune disorders in humans, dogs, and horses, particularly the condition systemic lupus erythematosus (SLE). The most commonly used method for detecting ANA in canine serum is the indirect immunofluorescence antibody assay (IFA) that detects dog IgG with reactivity towards mammalian cell nuclei. Interpretation of the IFA results is very subjective and dependent on the source of tissue/cellular substrate. We have developed a flow cytometry based assay to detect canine serum antibodies specific to histones. Histones were chosen as the target antigen because these nuclear proteins are the most common nuclear substrate for ANA in dogs with SLE. Microsphere beads were coated with histones and incubated with canine sera. Bound anti-histone antibodies were detected by FITC-conjugated rabbit $F(ab')_2$ anti-dog IgG. Sera from four groups of dogs (47 dogs total) were tested for anti-histone antibodies and compared with the traditional IFA assay. The groups included 15 healthy dogs, 15 dogs with noninflammatory diseases, 9 dogs with polyarthritis and positive ANA, and 8 German shepherds with perianal fistulas. The microsphere assay results indicated that only one dog in the noninflammatory group and four out of nine dogs in the polyarthritis group had mean fluorescent intensity values above our established cut-off (defined as 2 S.D. above the mean of healthy controls). There was moderate agreement between the anti-histone assay and the traditional ANA (kappa statistic = 0.54). Absorption of ANA positive serum with total histones dramatically diminished the fluorescent signal detected by flow cytometry and the speckled nuclear pattern observed by IFA, whereas preabsorption did not change the diffuse nuclear staining pattern. These findings indicate that the anti-histone assay will not replace the ANA test and that other nuclear proteins, such as ribonucleoproteins may contribute to the diffuse ANA patterns.

Keywords: Dog; Anti-histone antibodies; Anti-nuclear antibodies; Polyarthritis; Perianal fistulas; Flow cytometry

1. Introduction

Anti-nuclear antibodies (ANA) constitute a heterogeneous population of antibodies developed against nuclear components such as double stranded DNA

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(dsDNA), ribonuclear protein (RNP) and histones. In dogs with systemic lupus erythematosus (SLE), antibodies to histones are very common (61–74%) (Brinet et al., 1988), whereas the prevalence of anti-double stranded DNA is controversial (Monier et al., 1992; Shull et al., 1983; Thoren-Tolling and Ryden, 1991). Histones and DNA are the major components of the basic unit of chromatin referred to as the nucleosome. In dogs, the specificities of antibodies to histone fractions are H1, H2A, H3, and H4 (Monestier et al., 1995). Anti-nuclear antibodies including anti-histone antibodies are documented to be associated with several autoimmune diseases in humans (von Muhlen and Tan, 1995; Tan, 1989; Kubo et al., 1999). In dogs, ANA are mainly used as a major diagnostic indicator for SLE, however, ANA titers are of diagnostic significance in immune-mediated conditions such as connective tissue disorders, and polyarthritis (Bohnhorst et al., 2002; Hansson, 1999; Monestier et al., 1995).

At present the most commonly used assay to test for ANA in canine sera is the indirect immunofluorescence antibody assay (IFA) that utilizes rat liver sections or monolayers of epithelial cell cultures as the source of nuclear material (Chabanne et al., 1999a; Allbritton, 1993). There are several problems associated with the IFA test for ANA. The IFA is a qualitative assay using titration of the serum to determine the level of antibody. Anti-nuclear antibody assays are not standardized across veterinary laboratories and titers of ANA reported in normal individuals and diseased patients may vary widely from one laboratory to another (Allbritton, 1993). High titers may be observed in healthy dogs, reported more frequently when rat liver is used as the substrate than when monolayers of human epithelial-2 (HEp-2) cells are used (Hansson et al., 1996). Certain breeds of healthy dogs, particularly German shepherds, may have ANA titers as high as 1:4000 (Monier et al., 1992). Although the frequency is low, ANA can be identified in canine diseases other than SLE indicating the presence of ANA alone is not specific for autoimmune disease (Pedersen et al., 1976). Finally, the IFA test for ANA antibody targets the nucelosome and other nuclear components of the cell, rather than specific nuclear components.

Even though development of auto-antibodies to specific nuclear components in humans is associated with specific autoimmune disorders, canine ANA appear heterogeneous and little work has been done to identify disorders in dogs regarding production of autoantibodies to specific nuclear components. In humans, several ELISA based commercial kits are marketed in which a multitude of specific nuclear antigens coat the plate (Dahle et al., 2004; Tonuttia et al., 2004). The ELISA assays use extractable nuclear antigens which include ribonuclear proteins (RNP) and Smith (Sm) proteins. In human samples there is a good correlation with this assay to the IFA ANA assay when there is a homogenous or speckled pattern in the substrate (Tonuttia et al., 2004). In the case of canine SLE, dogs may have antibodies to Sm proteins and RNP, but previous investigations demonstrate that histones and a prominent canine autoantigen, a 43 kDa nuclear protein, are not included in the commercially available nuclear extract used for human sera (Welin et al., 1998). Therefore, extractable nuclear antigens used in these kits would not react with the majority of canine nuclear autoantibodies.

The purpose of this study is to report the development and evaluation of a novel microsphere flow cytometry based assay for detecting anti-histone antibody in canine sera from healthy dogs, dogs with noninflammatory conditions, German shepherds with perianal fistulas, and dogs with polyarthritis and positive ANA.

2. Methods and materials

2.1. Canine samples

Sera from a total of 47 dogs were tested. Groups of dogs consisted of 15 healthy dogs of various breeds, 8 German Shepherds with perianal fistulas, 15 dogs of various breeds with a variety of noninflammatory clinical conditions (renal failure, neoplasia, ocular disease, neurological disease, intervertebral disc disorder, unexplained anemia, malabsorption, and cranial cruciate rupture), and 9 dogs of various breeds with polyarthritis and positive ANA. The polyarthritis group of dogs was classified as ‘‘probable’’ SLE (PSLE) based on the presence of polyarthritis and positive ANA or identification of three diagnostic criteria specified by the 1982 revised American Rheumatism Association diagnostic criteria (Tan