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Research paper

Identification of specific antinuclear antibodies in dogs using a line immunoassay and enzyme-linked immunosorbent assay

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ABSTRACT

Circulating antinuclear antibodies (ANA) are commonly present in the systemic autoimmune disease Systemic Lupus Erythematosus (SLE) and in other systemic rheumatic diseases, in humans as well as in dogs. The indirect immunofluorescence (IIF)-ANA test is the standard method for detecting ANA. Further testing for specific ANA with immunoblot techniques or ELISAs is routinely performed in humans to aid in the diagnosis and monitoring of disease. Several specific ANA identified in humans have been identified also in suspected canine SLE but, in contrast to humans, investigation of autoantibodies in canine SLE is mainly restricted to the IIF-ANA test. Our aim was to identify both known and novel specific ANA in dogs and to investigate if different IIF-ANA patterns are associated with different specific ANA in dogs. Sera from 240 dogs with suspicion of autoimmune disease (210 IIF-ANA positive (ANA^{pos}) and 30 IIF-ANA negative (ANA^{neg})) as well as sera from 27 healthy controls were included. The samples were analysed with a line immunoassay, LIA (Euroline ANA Profile 5, Euroimmun, Lübeck, Germany) and four different ELISAs (Euroimmun). The ANA^{pos} dogs were divided in two groups depending on the type of IIF-ANA pattern. Of the 210 ANA^{pos} samples 68 were classified as ANA homogenous (ANA^{H}) and 141 as ANA speckled (ANA^{S}), one sample was not possible to classify. Dogs in the ANA^{H} group had, compared to the other groups, most frequently high levels of anti-double stranded deoxyribonucleic acid (dsDNA) and anti-nucleosome ANA. Anti-dsDNA antibodies were confirmed in some dogs with the *Crithidia luciliae* indirect immunofluorescence test (CLIFT). The frequency of ANA^{H} dogs with values above those observed in the healthy group was significantly higher compared to ANA^{S} dogs for anti-dsDNA, anti-nucleosome, and anti-histone reactivity. Dogs in the ANA^{S} group had, compared to the other groups, most frequently high levels of anti-ribonucleoproteins (RNP) and/or anti-Smith (Sm) antibodies. Reactivity against Sjögren's syndrome related antigens (SS)-A (including the Ro-60 and Ro-52 subcomponents), SS-B, histidyl tRNA synthetase (Jo-1), topoisomerase I antigen (Scl-70), polymyositis-scleroderma antigen (PM-Scl) and proliferating cell nuclear antigen (PCNA) was also noted in individual dogs. In conclusion, by using a commercial LIA and different ELISAs originally developed for detection of human ANA, we identified several specific ANA in serum samples from dogs sampled for IIF-ANA testing. Further, we found that the types of IIF-ANA pattern were associated with reactivity against some particular nuclear antigens.

Abbreviations: ANA, antinuclear antibodies; ANA^{H}, ANA homogenous; ANA^{neg}, IIF-ANA negative; ANA^{pos}, IIF-ANA positive; ANA^{S}, ANA speckled; CENP B, centromere protein B; CLIFT, *Crithidia luciliae* indirect immunofluorescence test; dsDNA, double stranded deoxyribonucleic acid; ENAs, extractable nuclear antigens; HEp-2, human epithelial-2; IIF, indirect immunofluorescence; Jo-1, histidyl tRNA synthetase; LIA, line immunoassay; M2, mitochondrial antigen 2; NSDTR, Nova Scotia Duck Tolling Retriever; PCNA, proliferating cell nuclear antigen; PM-Scl, polymyositis-scleroderma antigen; RIB, ribosomal P-protein; RNP, ribonucleoproteins; Scl-70, topoisomerase I; SLE, Systemic Lupus Erythematosus; Sm, Smith antigen; SS, Sjögren's syndrome related antigens.

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1. Introduction

Antinuclear antibodies (ANA) represent a heterogeneous group of autoantibodies directed at different nuclear antigens (Tan, 1989). Presence of high titres of ANA is a sensitive marker for the systemic autoimmune disease Systemic Lupus Erythematosus (SLE) in humans as well as in dogs (Tan et al., 1982; Bennett and Kirkham, 1987; Monier et al., 1992). Antinuclear antibodies, and some cytoplasmic autoantibodies, are also commonly present in other systemic rheumatic diseases (von Mühlen and Tan, 1995; Hansson-Hamlin et al., 2006; Hansson-Hamlin and Lilliehöök, 2009) as well as infrequently in healthy humans and dogs (Bennett and Kirkham, 1987; Tan et al., 1997). Positive titres of ANA can sometimes also be detected in canine leishmaniasis (Lucena et al., 1996), a disease that is rare in Sweden. The standard method for detecting ANA is indirect immunofluorescence; IIF (Coons et al., 1950; Tan et al., 1982; Bennett and Kirkham, 1987). In human diagnostics, human epithelial-2 (HEp-2) cells are commonly used as substrate (von Mühlen and Tan, 1995). In veterinary diagnostics, IIF-ANA test with HEp-2 cells are also used in the diagnosis of canine SLE and canine SLE-related disorders (Hansson et al., 1996; Bell et al., 1997).

Antinuclear antibodies are directed against several different antigens or groups of antigens such as double stranded deoxyribonucleic acid (dsDNA), histones, Smith antigen (Sm), ribonucleoproteins (RNP), Sjögren's syndrome related antigens; (SS)-A/Ro, and SS-B/La (Tan, 1989). Several specific ANA previously identified in humans like the anti-histone (Costa et al., 1984; Monier et al., 1992), anti-RNP (Monier et al., 1978, 1980; Costa et al., 1984; Thoren-Tolling and Ryden, 1991; Fournel et al., 1992; Welin Henriksson et al., 1998; Hansson-Hamlin and Rönnelid, 2010), anti-Sm (Monier et al., 1978; Costa et al., 1984; Hubert et al., 1988; Fournel et al., 1992), anti-SS-A (Monier et al., 1988; Fournel et al., 1992), and anti-SS-B (Monier et al., 1992) have been identified also in suspected canine SLE cases. In addition, specific ANA against a 43 kDa protein known as the hnRNP G and against an unidentified antigen called type-2 antigen have been identified in dogs but not in humans (Costa et al., 1984; Soulard et al., 1991; Fournel et al., 1992). Anti-dsDNA antibodies in canine SLE have only infrequently been reported by some authors (Fournel et al., 1992; Monier et al., 1992).

Different IIF patterns are indicative of specific ANA (von Mühlen and Tan, 1995). Two common IIF patterns in humans are the homogenous pattern and the speckled pattern. Other patterns are also recognised. A homogenous pattern is mainly associated with reactivity against dsDNA and DNA associated proteins while a speckled pattern is mainly associated with specific ANA against RNP, Sm, SS-A, SS-B; also called extractable nuclear antigens; ENAs (Sharp et al., 1972; Tozzoli et al., 2002). The IIF-pattern and specific ANA can also be associated with different autoimmune disorders, some with high specificity for a particular disease (von Mühlen and Tan, 1995). For example, the anti-dsDNA and anti-Sm antibodies are quite specific for human SLE, and included in the diagnostic criteria for human SLE (Tan et al., 1982; Hochberg, 1997). Some specific ANA, like the anti-dsDNA antibodies, are associated with particular symptoms and can correlate with disease activity (Koffler et al., 1967; Swaak et al., 1986; Terborg et al., 1990). Hence, testing for specific ANA is an aid in the diagnosis and monitoring of autoimmune diseases in humans. Commonly used methods to screen for specific ANA in humans are ELISA and immunoblot techniques (Tozzoli et al., 2002; Murdjeva et al., 2011). In order to investigate anti-dsDNA antibodies, the *Crithidia luciliae* indirect immunofluorescence test (CLIFT) is also a commonly used method (Aarden et al., 1975).

In contrast to humans where testing for specific ANA is routinely performed in suspected SLE cases, investigation of autoantibodies in suspected canine SLE is mainly restricted to the IIF-ANA test (Bennett, 1987). The IIF-ANA test is almost always positive in canine SLE, but a positive test alone is not sufficient for the diagnosis. Therefore, other clinical, haematological and/or biochemical alterations also have to be present (Bennett, 1987). In dogs, two distinct immunofluorescence patterns can be identified when using HEp-2 cells as a substrate (Fig. 1), the homogenous pattern, with chromosomal staining of mitotic cells and the speckled pattern, with non-chromosomal staining (Hansson and Karlsson-Parra, 1999). The different patterns have been correlated with clinical signs in dogs, suggesting that dogs with a homogenous pattern more often show involvement of several organ systems than dogs with a speckled pattern (Hansson-Hamlin et al., 2006).

Today, even though there is some knowledge about specific ANA in dogs, previous studies show that more research is needed in the field. The occurrence of some specific ANA that are of importance in human medicine has not been investigated in dogs. Unidentifiable ANA have also been found in dogs (Monier et al., 1980; Costa et al., 1984; Hansson and Karlsson-Parra, 1999). Further, very little is known about the clinical and pathological role of specific ANA in dogs. In the present study we investigated sera from IIF-ANA tested dogs for specific ANA reactivity against several antigens known to be associated with systemic autoimmune or rheumatic disease in humans. Our aim was to identify both known and novel specific ANA in dogs and to investigate if different IIF-ANA patterns are associated with different specific ANA in dogs.

2. Materials and methods

2.1. Study design and study population

Included in the study were 210 IIF-ANA positive (ANA^{pos}) and 30 IIF-ANA negative (ANA^{neg}) dog sera. The samples had been submitted to the Clinical Pathology Laboratory, University Animal Hospital, Uppsala, Sweden, from veterinarians all over Sweden for routine IIF-ANA testing during May 2002–June 2012. Eight of the dogs had been resampled 35–480 days from the first sampling occasion, so for these dogs two serum samples were available. For the majority of dogs included in the study, clinical information was not available, but the ANA^{pos} as well as the ANA^{neg} dogs were assumed to have a suspicion of autoimmune disease since the samples had been sent for IIF-ANA testing.

The sera had been stored in −20 or −70 °C until analysis and had been through two to four thaw–freeze cycles. The serum samples were sent to Euroimmun, Lübeck, Germany, where the IIF-ANA tests were repeated. In order to detect specific ANA, two different assays were performed, a line immunoassay (LIA) and an ELISA. As a healthy control group, sera from 27 blood donor dogs and laboratory dogs from Germany were also analysed. No information about sex and breed was available for these dogs. In total, 275 serum samples from 267 dogs were investigated for IIF-ANA and for the presence of specific ANA.

Confirmatory analyses of ds-DNA reactivity with CLIFT were performed in 39 of the diseased dog sera as well as in 12 healthy control sera (collected from the Canine Biobank, SLU, Uppsala, ethical permission C2/12).

The blood samples from the diseased dogs were initially taken for diagnostic purposes (IIF-ANA tests) and for health screening for the control dogs. An ethical permission was obtained from the local ethical committee, Uppsala, Sweden (C 418/12).

2.2. IIF-ANA test

IIF-ANA tests were performed at the Clinical Pathology Laboratory, University Animal Hospital, Swedish University of Agricultural