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Autoantibodies to histone, DNA and nucleosome antigens in canine systemic lupus erythematosus

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SUMMARY

Dogs can develop systemic lupus erythematosus syndromes that are clinically similar to those seen in humans. In contrast, previous observations suggest differences in their autoantibody reactivity patterns against histones and DNA which are components of the nucleosome in chromatin. The objective of this study was to assess comprehensively the levels of autoantibodies against histone, DNA and nucleosome antigens in a population of lupus dogs. The specificities of antibodies in lupus and control dog sera were determined using IgM- and IgG-specific reagents in an ELISA against a variety of chromatin antigens. When compared with control sera, IgG antibodies to individual histones H1, H2A, H3 and H4 were significantly higher in the lupus group. In contrast, we did not detect IgG antibodies specific for H2B, H2A-H2B, DNA, H2A-H2B-DNA or nucleosomes in lupus dogs. There was no significant increase in any of the IgM specificities tested. Therefore, the reactivity pattern to nucleosome antigens in canine lupus is restricted to IgG antibodies against individual histones H1, H2A, H3 and H4. This stands in contrast with human and murine lupus, where autoantibodies are directed against a wide variety of nucleosomal determinants, suggesting that unique mechanisms lead to the expansion of anti-histone antibody clones in canine lupus. The high incidence of glomerulonephritis in dog lupus suggests that anti-DNA antibodies are not required for the development of this complication, whereas IgG anti-histone antibodies may be relevant to its pathogenesis.

Keywords antinuclear antibodies histones nucleosomes canine lupus

INTRODUCTION

Animal models are invaluable to help us understand the pathogenesis of human diseases. A great part of our knowledge about the physiopathology of systemic lupus erythematosus (SLE) comes from the study of several mouse strains that spontaneously develop a lupus-like disease [1]. The dog is another mammalian species to present with SLE [2–4]. The canine lupus model is particularly interesting because of its clinical similarity with human SLE. Frequent manifestations in canine lupus include fever, polyarthritis, glomerulonephritis, mucocutaneous lesions and adenosplenomegaly [5]. Like human SLE, canine lupus is a chronic disease with alternating subacute periods and relapses. In contrast, such a cyclic evolution is not observed in lupus mice, where the disease steadily progresses to its terminal stage [1].

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As in human SLE, most lupus dogs produce antinuclear antibodies detectable by immunofluorescence. These include autoantibodies to histones, Sm, RNP antigens and other specificities encountered in human lupus [6–8]. Autoantibodies to native DNA seem less frequent in canine SLE than in human or murine lupus, where they are the hallmark of the disease. The prevalence of anti-DNA in canine lupus is controversial, since some authors have reported an almost complete lack of anti-DNA antibodies, whereas others claim that they are detectable [6,8–12]. DNA and histones are the components of the nucleosome, the basic unit of chromatin [13]. The nucleosome is composed of a central tetramer of two molecules each of histones H3 and H4 flanked by two dimers of histones H2A and H2B, and surrounded by about 160 base pairs of DNA. Histone H1 is located external to the nucleosome at the entry and exit points of the DNA. Recently, it became apparent that antinuclear antibodies in human and murine lupus can distinguish complex epitopes that result from the ordered interactions between histones and DNA [14,15]. An epitope frequently recognized is composed of the histone H2A-

H2B dimer and of DNA. MoAbs to H2A-H2B-DNA or to more complex nucleosome epitopes have been isolated from spontaneously autoimmune mice [16,17]. The characterization of these MoAbs has shown that overlaps exist between some anti-DNA and anti-histone specificities. For instance, MoAbs to H2A-H2B-DNA can also react, albeit to a lesser extent, with the H2A-H2B dimer in the absence of DNA, and with DNA in the absence of histones [18].

In this study of a canine SLE population, our overall aim was to assess concurrently in a quantitative ELISA the levels of the various autoantibodies directed against histone, DNA and nucleosome epitopes. We have included antigens that were not previously evaluated such as H2A-H2B, H2A-H2B-DNA and nucleosomes. Beforehand, reactivity against individual histones was only detected qualitatively by immunoblotting [7]. In the present study, we used purified individual histones as antigens in the ELISA assay. Moreover, we also analysed autoantibody specificities using IgM- and IgG-specific reagents, whereas earlier studies have used detection reagents that were not isotype-specific.

MATERIALS AND METHODS

Animals

We used sera from 43 SLE dogs and 20 healthy dogs. All animals were privately owned and seen in consultation at the Ecole Nationale Vétérinaire de Lyon. Diagnosis of SLE required fulfillment of at least four of the American Rheumatism Association criteria for human SLE. All SLE dog sera were positive for the detection of antinuclear antibodies by immunofluorescence [5].

ELISA

The assays for the detection of canine antibodies to histone, DNA and nucleosome epitopes were adapted from methods used with human and murine antibodies [16,17,19]. The antigens, individual bovine histones, H2A-H2B dimer, nucleosomes and native DNA were prepared as previously described [16,17,20]. Histones H1, H2A, H2B, H3, H4 and H2A-H2B were directly adsorbed onto ELISA plates at 2·5 μg/ml in carbonate buffer. The H2A-H2B-DNA complex was reconstituted by adding DNA (2·5 μg/ml in PBS) for 30 min to ELISA plates already coated with H2A-H2B [16]. For anti-DNA and anti-nucleosome assays, DNA or nucleosomes (2·5 μg/ml in PBS) were added for 30 min to poly-L-lysine-coated ELISA plates [17]. For these two antigens, plates coated with poly-L-lysine only without DNA or histones were used to determine background reactivities. All antigen-coated plates were washed with PBS containing 0·05% Tween-20 (PBS-T) and blocked with PBS containing 1% bovine serum albumin (BSA). Dog sera, diluted 1:200 in PBS-BSA-T, were then added for 2 h to the ELISA plates. Binding was revealed with peroxidase-goat anti-dog IgM or anti-IgG conjugates (Kierkegaard & Perry Laboratories, Gathersburg, MD) at the dilution recommended by the manufacturer (1:500 in PBS-BSA-T). After washing, 150 μl of substrate (H_2O_2, 0·012%; *o*-phenylenediamine, 4 mg/ml) were added to each well and the optical densities were read at 450 nm after 20 min using a kinetic microplate reader (Molecular Devices, Menlo Park, CA). For the antigens directly adsorbed onto the ELISA plates (individual histones, H2A-H2B and H2A-H2B-DNA), the results were directly expressed as means of optical densities. For antigens bound to poly-L-lysine-coated plates (nucleosomes and DNA), optical density values obtained with plates coated only with poly-L-lysine were subtracted from those obtained with nucleosome- or DNA-coated plates. Comparisons between SLE and control groups were performed with a one-way analysis of variance using a one-tailed *F*-test.

RESULTS

The sera from 43 SLE dogs and 20 control animals were tested by ELISA for their IgM and IgG reactivities against various chromatin antigens. These antigens include the five individual histones, DNA, nucleosomes, the H2A-H2B and H2A-H2B-DNA complexes (Table 1). None of the IgM reactivities was elevated in SLE dogs compared with control animals. In contrast, IgG antibodies to individual histones H1, H2A, H3

Table 1. Levels of IgM and IgG antibodies to histone, DNA and nucleosome antigens in the sera of systemic lupus erythematosus (SLE) and control dogs

	IgM		IgG	
Antigen	Controls $n = 20$	SLE $n = 43$	Controls $n = 20$	SLE $n = 43$
H1	0·85 ± 0·40	0·90 ± 0·31	0·21 ± 0·15	0·54 ± 0·36**
H2A	0·37 ± 0·12	0·32 ± 0·11	0·14 ± 0·12	0·34 ± 0·34*
H2B	0·49 ± 0·20	0·38 ± 0·17	0·39 ± 0·38	0·21 ± 0·23
H3	0·55 ± 0·18	0·43 ± 0·21	0·09 ± 0·08	0·35 ± 0·34**
H4	0·84 ± 0·20	0·69 ± 0·20	0·22 ± 0·10	0·56 ± 0·35**
H2A-H2B	0·39 ± 0·14	0·32 ± 0·14	0·04 ± 0·09	0·29 ± 0·44*
H2A-H2B-DNA	0·35 ± 0·14	0·30 ± 0·15	0·12 ± 0·12	0·40 ± 0·46**
Nucleosome	0·02 ± 0·05	0·01 ± 0·05	0·01 ± 0·02	0·13 ± 0·35
DNA	0·24 ± 0·14	0·12 ± 0·13	0·01 ± 0·04	0·01 ± 0·05

Results are expressed as means of optical densities ± s.d.
* $P < 0.05$ *versus* controls; ** $P < 0.01$ *versus* controls.

and H4 (but not H2B) were increased in SLE dogs. The lack of reactivity to H2B was also observed in a previous study that used an immunoblotting technique to detect anti-histone antibodies [7]. In contrast, H2B is a frequent target for autoantibodies in both human and murine lupus [21,22]. The IgG reactivities to H2A-H2B and H2A-H2B-DNA from SLE animals were also significantly higher than those of the controls. On the other hand, we did not find a significant increase in anti-nucleosome or anti-DNA antibodies among SLE dogs.

The IgG binding patterns of several representative SLE sera are depicted in Fig. 1. For most animals, the IgG reactivities to various histone molecules were proportionally elevated, i.e. a serum that strongly reacts with H1 will also strongly bind to H2A, H3 and H4 (Fig. 1). It is also important to observe that the levels of IgG antibodies to H2A, H2A-H2B or H2A-H2B-DNA were approximately equivalent in individual animals (Fig. 1). Hence, in SLE dogs, the mean levels of IgG antibodies to H2A-H2B or to H2A-H2B-DNA were not significantly greater than those of antibodies to individual H2A (Table 1). Therefore, most of the observed reactivity to H2A-H2B or H2A-H2B-DNA did not result from antibodies specific for these multimolecular structures, but was merely a consequence of the presence of antibodies of H2A.

Several studies have previously indicated the absence of anti-DNA antibodies in canine SLE. Likewise, we did not detect any reactivity to DNA in our panel of SLE dog sera. A possible concern was the presence in normal dog serum of a DNA-binding protein that could have blocked autoantibody reactivity [23]. This protein is found in several species other than dog (horse, sheep, cow, cat, mink), but is absent in primate, rabbit or mouse sera [23]. To our knowledge, this protein has not been further characterized since its original description in 1972 [23], although it has been reported to interfere in the Farr assay [3,24]. To investigate the possible

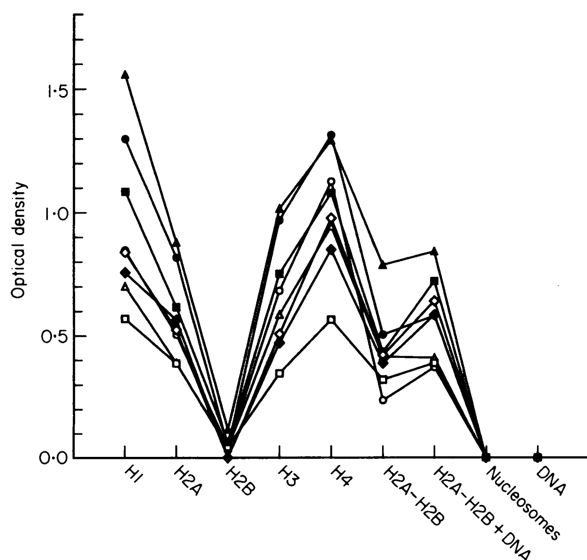

Fig. 1. IgG reactivities to histone, DNA and nucleosome antigens of several representative canine systemic lupus erythematosus (SLE) sera. Results are expressed as optical densities. ○, Dog 25; ●, dog 26; △, dog 34; ▲, dog 38; □, dog 43; ■, dog 44; ◇, dog 46; ◆, dog 47.

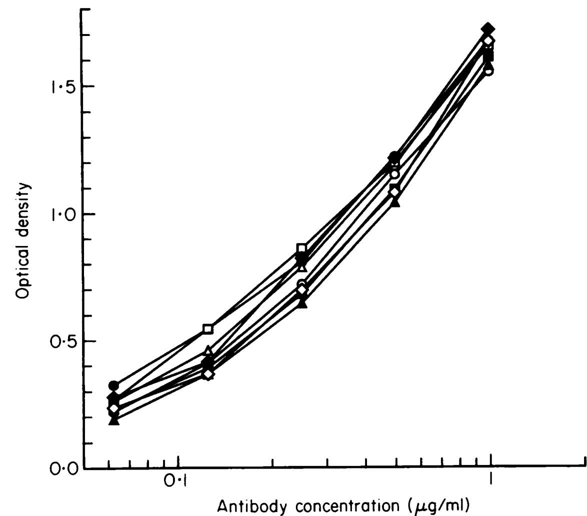

Fig. 2. DNA-binding activity of PL9-10, a murine anti-DNA MoAb, after dilution in buffer containing 0·5% serum from various species. The ELISA was conducted as described in Materials and Methods for canine anti-DNA antibodies, except that the peroxidase–anti-dog IgG conjugate was replaced with a peroxidase–anti-mouse IgG conjugate. Results are expressed as optical densities. See Results for further description. Serum: ○, nil; ●, dog; △, mouse; ▲, human; □, rat; ■, goat; ◇, horse; ◆, hamster.

role of this protein, we tested in our anti-DNA assay PL9-10, a murine anti-DNA MoAb [18]. PL9-10 was diluted in PBS-BSA-T containing 0·5% serum from several species, including dog. These experimental conditions mimic those used in the present study of canine SLE sera. The results in Fig. 2 indicate that none of the sera interfered with the PL9-10 anti-DNA reactivity, ruling out a non-specific inhibitory role for dog serum.

In conclusion, the levels of IgG antibodies to individual histones H1, H2A, H3 and H4 were increased in canine SLE, but no antibodies to DNA, nucleosomes, H2A-H2B or H2A-H2B-DNA were detectable by ELISA in the sera of these animals.

DISCUSSION

One of the most unique features of canine SLE is the paucity of detectable anti-DNA antibodies as described in the present and previous studies [3,6,8]. Although some authors have observed DNA-reactive antibodies in dog lupus sera [9–12], they often recognize that their levels are lower than those found in human disease. Various factors, such as differences in dog populations or in diagnostic criteria, could be responsible for these discrepancies. In addition, some of these studies may not have used highly purified native DNA. The use of mammalian DNA that still contains histone contaminants or single-stranded DNA would result in false positive results, since many SLE dog sera contain anti-histone or anti-single-stranded DNA antibodies [8]. Regardless, the technique that we used in this study is quite standard for human and mouse anti-DNA antibodies, and would have allowed the detection of anti-DNA antibodies if they were present in our group of SLE dogs.

Canine SLE sera are also notable by the absence of antibodies specific for nucleosomes or histone–DNA com-

plexes. The concurrent lack of autoantibodies to DNA and to nucleosomal antigens is not by itself surprising, since these two reactivities share several features, such as an overlap in their specificities. For instance, autoantibodies to H2A-H2B-DNA can frequently bind to DNA in the absence of histones [18]. Antibodies reactive with DNA or with DNA–histone complexes have common genetic characteristics that include recurrent usage of certain V genes and unusual V(D)J rearrangements [16,17,25]. Some of these features, as well as the somatic mutation process, result in the presence of arginine or asparagine residues which are critical for binding to DNA by itself or to the DNA moiety of nucleosome epitopes [25].

In contrast to anti-DNA antibodies, autoantibodies to several individual histones are elevated in canine SLE. Current views of the pathogenesis of systemic autoimmunity support a role for nucleosomes as autoimmunogenic particles during SLE [14,26]. If this is the case, why are only anti-histone antibodies produced in dog SLE, in contrast to mice and humans, where autoantibodies are directed against a diverse array of nucleosomal epitopes? If seems unlikely that the genetic mechanisms that give rise to DNA-reactive antibodies in other species would be lacking in dogs, since some of these mechanisms, such as V gene somatic hypermutation, apparently exist in all mammals. It is, however, plausible that the DNA-binding protein which is found in dog serum (but not in mice or humans) plays a role in this process [23]. Following extracellular release of nucleosomes in lupus-prone animals, this protein could combine with them and mask DNA-containing epitopes. This protein may also displace histones from the nucleosomes and reveal cryptic epitopes on the histone molecules. It is noteworthy that the pattern of reactivity to individual histones in canine SLE differs from that observed in human SLE. Anti-histone antibodies in human and murine lupus are directed against the accessible trypsin-sensitive regions of the histones [27,28], whereas antibodies in canine SLE react with their trypsin-resistant regions which are normally inaccessible inside the nucleosome [7]. Therefore, the mechanisms leading to the production of anti-chromatin antibodies during dog lupus may be different from those in mouse and human disease. Additional characterization of the DNA-binding protein present in dog serum could further our understanding of the pathogenesis of canine SLE.

In contrast to the antinuclear antibody pattern, the clinical manifestations of canine SLE are very similar to those observed in human patients. As in humans, renal involvement is frequent, with an incidence estimated at 65% [5]. In humans and in mice, autoantibodies can be found in glomerular deposits and probably play a role in lupus nephritis, although the pathogenesis of this complication is not completely understood. Anti-DNA antibodies may contribute to the disease by forming immune complexes with nuclear antigens [29] or by binding directly to the glomerular basement membrane [30]. This study, as well as several previous reports [3,6,8], indicates however that lupus nephritis can develop in the absence of anti-DNA antibodies. The view that anti-DNA antibodies are predominantly responsible for lupus nephritis in humans has indeed been challenged [31], and alternative mechanisms involving histones have been proposed [32]. Cationic histones can bind directly to the negatively charged glomerular basement membrane and act as planted antigens for antibody binding [33]. Histones and anti-histone antibodies have indeed been eluted from glomerular deposits in autoimmune mice [34,35]. The high incidence of renal lesions in canine SLE may therefore be related to the presence of pathogenic IgG anti-histone antibodies in lupus dogs.

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REFERENCES

1 Theofilopoulos AN, Dixon FJ. Murine models of systemic lupus erythematosus. Adv Immunol 1985; **37**:269–390.

2 Lewis RM, Schwartz R, Henry Jr WB. Canine systemic lupus erythematosus. Blood 1965; **25**:143–60.

3 Monier JC, Dardenne M, Rigal D *et al.* Clinical and laboratory features of canine lupus syndromes. Arthritis Rheum 1980; **23**:294–301.

4 Jones DRE. Canine systemic lupus erythematosus: new insights and their implications. J Comp Pathol 1993; **108**:215–28.

5 Fournel C, Chabanne L, Caux C *et al.* Canine systemic lupus erythematosus. I: a study of 75 cases. Lupus 1992; **1**:133–9.

6 Costa O, Fournel C, Lotchouang E *et al.* Specificities of antinuclear antibodies detected in dogs with systemic lupus erythematosus. Vet Immunol Immunopathol 1984; **7**:369–82.

7 Brinet A, Fournel C, Faure JR *et al.* Anti-histone antibodies (ELISA and immunoblot) in canine systemic lupus eythematosus. Clin Exp Immunol 1988; **74**:105–9.

8 Monier JC, Ritter J, Caux C *et al.* Canine systemic lupus erythematosus. II: antinuclear antibodies. Lupus 1992; **1**:287–93.

9 Shull RM, Miller HA, Chilina AR. Investigation of the nature and specificity of antinuclear antibody in dogs. Am J Vet Res 1983; **44**:2004–8.

10 Bennett D, Kirkham D. The laboratory identification of serum antinuclear antibody in the dog. J Comp Pathol 1987; **97**:523–39.

11 Zouali M, Migliorini P, Mackworth-Young CG *et al.* Nucleic acid-binding specificity and idiotypic expression of canine anti-DNA antibodies. Eur J Immunol 1988; **18**:923–7.

12 Thoren-Tolling K, Ryden L. Serum autoantibodies and clinical/pathological features in German shepherd dogs with a lupuslike syndrome. Acta Vet Scand 1991; **32**:15–26.

13 van Holde KE. Chromatin. In: Rich A, ed. Springer series in molecular biology. New York: Springer-Verlag, 1989.

14 Burlingame RW, Rubin RL, Balderas RS *et al.* Genesis and evolution of antichromatin autoantibodies in murine lupus implicates T-dependent immunization with self antigen. J Clin Invest 1993; **91**:1687–96.

15 Burlingame RW, Boey ML, Starkebaum G *et al.* The central role of chromatin in autoimmune responses to histones and DNA in systemic lupus erythematosus. J Clin Invest 1994; **94**:184–92.

16 Losman MJ, Fasy TM, Novick KE *et al.* Monoclonal autoantibodies to subnucleosomes from a MRL/MP-+/+ mouse. Oligoclonality of the antibody response and recognition of a determinant composed of histones H2A, H2B and DNA. J Immunol 1992; **148**:1561–9.

17 Losman JA, Fasy TM, Novick KE *et al.* Nucleosome-specific antibody from an autoimmune MRL/Mp-*lpr*/*lpr* mouse. Arthritis Rheum 1993; **36**:552–60.

18 Losman MJ, Fasy TM, Novick KE *et al.* Relationships among antinuclear antibodies from autoimmune MRL mice reacting with histone H2A-H2B dimers and DNA. Int Immunol 1993; **5**:513–23.

19 Monestier M, Losman JA, Fasy TM *et al.* Anti-histone antibodies in antinuclear antibody positive juvenile arthritis. Arthritis Rheum 1990; **33**:1836–41.

20 Monestier M, Fasy TM, Böhm L. Monoclonal anti-histone H1 autoantibodies from MRL *lpr*/*lpr* mice. Mol Immunol 1989; **26**:749–58.

21 Monestier M, Kotzin BL. Antibodies to histones in systemic lupus erythematosus and drug-induced lupus syndromes. Rheum Dis Clin North Am 1992; **18**:415–36.
22 Rubin RL. Antihistone antibodies, systemic lupus erythematosus, 2nd edn. Lahita RG, ed. New York; Churchill Livingstone Inc, 1992.
23 Thoburn R, Hurvitz AI, Kunkel HG. A DNA-binding protein in the serum of certain mammalian species. Proc Natl Acad Sci USA 1972; **69**:3327–30.
24 Zeromski J, Thoren-Tolling K, Bergqvist R *et al.* DNA binding proteins in canine sera. A method for removal of nonspecific DNA binding in the Farr assay. Vet Immunol Immunopathol 1984; 7:169–83.
25 Radic MZ, Weigert M. Genetic and structural evidence for antigen selection of anti-DNA antibodies. Annu Rev Immunol 1994; **12**:487–520.
26 Rumore PM, Steiman CR. Endogenous circulating DNA in systemic lupus erythematosus. Occurrence as multimeric complexes bound to histone. J Clin Invest 1990; **86**:69–74.
27 Portanova JP, Arndt RE, Tan EM *et al.* Anti-histone antibodies in idiopathic and drug-induced lupus recognize distinct intrahistone regions. J Immunol 1987; **138**:446–51.
28 Portanova JP, Arndt RE, Kotzin BL. Selective production of autoantibodies in graft-*versus*-host-induced and spontaneous murine lupus: predominant reactivity with histone regions accessible in chromatin. J Immunol 1988; **40**:755–60.
29 Lambert PH, Dixon FJ. Pathogenesis of the glomerulonephritis of NZB/W mice. J Exp Med 1968; **127**:502–22.
30 Madaio MP, Carlson J, Cataldo J *et al.* Murine monoclonal anti-DNA antibodies bind directly to glomerular antigens and form immune deposits. J Immunol 1987; **138**:2883–9.
31 Brinkman K, Termaat R, Berden JHM *et al.* Anti-DNA antibodies and lupus nephritis: the complexity of crossreactivity. Immunol Today 1990; **11**:232–4.
32 Kramers K, Hylkema M, Termaat R-M *et al.* Histones in lupus nephritis. Exp Nephrol 1993; **1**:224–8.
33 Schmiedeke TM, Stockl FW, Weber R *et al.* Histones have high affinity for the glomerular basement membrane. Relevance for immune complex formation in lupus nephritis. J Exp Med 1989; **169**:1879–94.
34 Schmiedeke TM, Stockl FW, Muller S *et al.* Glomerular immune deposits in murine lupus models may contain histones. Clin Exp Immunol 1992; **90**:453–8.
35 Termaat R-M, Assmann KJM, van Son JPHF *et al.* Antigen-specificity of antibodies bound to glomeruli of mice with systemic lupus erythematosus-like syndromes. Lab Invest 1993; **68**:164–73.