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Antinuclear Antibodies (ANA) in Gordon Setters with Symmetrical Lupoid Onychodystrophy and Black Hair Follicular Dysplasia

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Øvrebø Bohnhorst J, Hanssen I, Moen T: Antinuclear Antibodies (ANA) in Gordon setters with symmetrical lupoid onychodystrophy and black hair follicular dysplasia. Acta vet. scand. 2001, 42, 323-329. – Antinuclear antibodies (ANA) were demonstrated in 3 out of 10 Gordon setters with symmetrical lupoid onychodystrophy and in 5 out of 13 Gordon setters with black hair follicular dysplasia. Two dogs showed both symmetrical lupoid onychodystrophy and black hair follicular dysplasia, and one of these was ANA positive. The results suggest that symmetrical lupoid onychodystrophy and black hair follicular dysplasia in the Gordon setter might be autoimmune diseases that are pathogenetically related, which might indicate a common genetic predisposition.

Introduction

During the last decade there has been an increasing incidence of claw disease in dogs of the Gordon setter breed in Norway. The affected dogs show sudden pain and lameness and are observed to be licking 1 or more toes. By inspecting the feet it becomes evident that 1 or more, and eventually all claws are detaching. Secondary bacterial infections are common.

Histopathological studies of this phenomenon have not been conducted in Norway, but Jønsson (unpubl. 1996) found vacuolar alteration and degeneration of epidermal basal cells, and acute and chronic inflammation and pigmentary incontinence in the dermis of the toes of an affected Swedish Gordon setter. These findings are in accordance with symmetrical lupoid onychodystrophy (*Scott et al.* 1995 a).

The dogs have been treated with antibiotics, glucocorticoids, zinc and fatty acid supplementation, and the response has been recorded from poor to good: Some dogs are put to death because of chronic pain, but most dogs go on living in a state of chronic onychodystrophy where every claw is misshapen, with stunted friable structures (Fig. 1). A few dogs recover, but acute relapses are common.

Extensive genetic analyses have not yet been conducted, but pedigrees of 56 cases gathered since 1977 show that these dogs can be traced back to common ancestors.

During the same period dogs have been frequently observed among Norwegian Gordon setters that abruptly start shedding their black hairs, without normal regrowth taking place. This most often happens when the dogs are between 1 and 2 years old, but sometimes even earlier. Afterwards they appear with a thin hair coat composed either of thin wooly hairs that are easily removed (Fig. 2), or by short stiff hairs (Fig. 3). The changes are most evident on

the trunk caudal to the shoulders. The head, neck and legs are in most dogs normally coated.The degree of changes varies from slight in some dogs to almost alopecic in others. The skin is slightly pigmented in affected areas. Tan coloured areas are never affected. The owners report that the claws grow slowly in these dogs. Treatment with vitamin B complex and fatty acid supplementation has been tried without obvious effect.

The aim of this study was to investigate whether these dogs had signs of systemic autoimmunity. The antinuclear antibody (ANA) test is currently considered the most specific and sensitive serologic test for systemic lupus erythematosus (*Monier et al.* 1992, *Scott et al.* 1995 a). That the claw disease in a Swedish Gordon setter seemed to be of lupoid character, and our suspicion that black hair follicular dysplasia and symmetrical lupoid onychodystrophy in the Gordon setter might somehow be connected, were the incitaments for investigating the occurence of ANA in Gordon setters with symmetrical lupoid onychodystrophy and black hair follicular dysplasia, respectively.

Materials and methods

Animals

The animals studied comprised 21 healthy Gordon setters (controls) and 21 Gordon setters with symmetrical lupoid onychodystrophy and/or black hair follicular dysplasia, respectively.

As controls were chosen dogs brought to the clinic for vaccinations. The group comprising symmetrical lupoid onychodystrophy consisted of dogs that all were in the acute phase of detaching several claws, while the the black hair follicular dysplasia group were dogs that presented typical clinical signs of this disease, and in most instances had done so for a long while. Two dogs showed both symmetrical lupoid onychodystophy and black hair follicular dysplasia, while 1 dog with symmmetrical lupoid onychodystrophy and 1 dog with black hair follicular dysplasia in addition had muscular pain that could not be attributed to trauma.

The diagnoses were based on clinical findings, verified by histopathological investigations for 3 dogs in each of the main disease groups. Histhopathological investigations were performed on entire toes and skin biopsies taken from the flank, fixed in buffered 4% formaldehyd. The toes were first decalcified in a mixture of nitric and sulphuric acid. All specimens were embedded in paraffin and sections were stained in haematoxylin and eosin.

Blood samples were collected from the cephalic vein and serum prepared and frozen at –20 °C for later testing of antibodies.

Serum analyses

The methods applied for detection of canine autoantibodies were locally modified variants of routine human diagnostical techniques and established as part of a C. Sc. dissertation (unpublished). The techniques were worked out by use of a collection of 500 sera from dogs of different breeds with a variety of symptoms of mainly rheumatic, autoimmune and febrile disease conditions and with 45 sera from healthy dogs as controls. The sera were partly collected locally and partly provided by Kjerstin Thoren-Tolling and Solveig Knagenhjelm, The Norwegian College of Veterinary medicine, Oslo, Norway.

Antinuclear antibodies (ANA) were detected by use of the indirect immunofluorescnce (IIF) technique using Hep-2 cells fixed in alcohol as antigen substrate (*Miller et al.* 1985).The cells were cultivated in the laboratory and dispersed into Terasaki plates for application in the test.

The sera were screened for ANA reactivity at a 1:20 dilution in PBS and the reaction visualised by a FITC conjugated Fc- specific goat anti-dog IgG (Cappel research Products, Durham, NC)

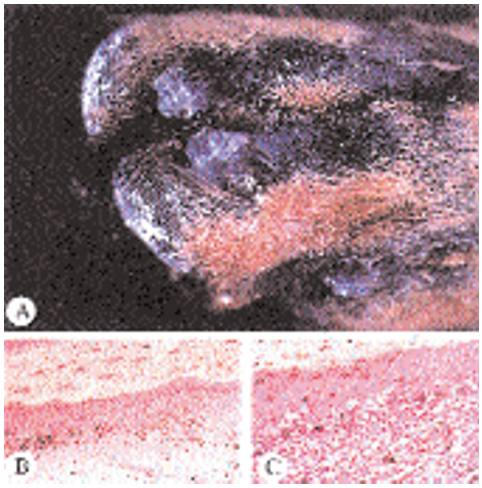

Figure 1 A. Paw of a Gordon setter with chronic symmetrical lupoid onychodystrophy showing small, stunted claws. B and C are10x and 40x objective lens pictures , respectively, from the clawbed of the same paw exhibiting histopathological features of lichenoid infiltrate of mononuclear cells at the dermo/epidermal junction, hydropic degeneration and apoptosis of individual keratinocytes in the basal layer, and marked pigmentary incontinence H&E.

Figure 2. Gordon setter with strong degree and typical distribution of black hair follicular dysplasia.

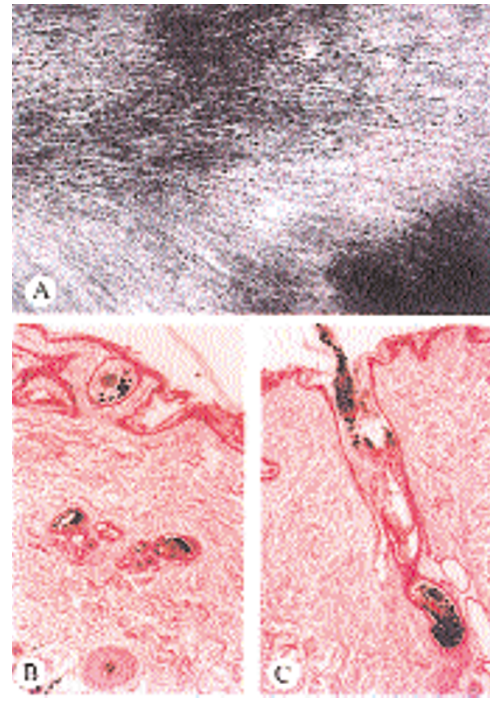

Figure 3 A. Flank of a Gordon setter with marked black hair follicular dysplasia. The same dog had also symmetrical lupoid onychodystrophy. B and C show histopathological sections, 10x objective, of A. There are irregular clumping of pigment in hair shafts, malformed hairs in pilar canals, and melanin in macrophages around the base of some follicles. H&E.

at dilution 1:60. The serum dilution 1:20 was chosen on the basis of positive reactions in 70/230 sera (31.3%) from dogs mainly with signs of systemic disease and 0/45 sera from healthy controls, both groups comprising different breeds. This corresponds well with what has been published by *Hansson et al.* 1996.

Screening for antibodies against extractable nuclear antigens (ENA) was done by 2 methods displaying partly overlapping results. One technique used immunelectrophoresis in agarose gel with calf thymus extract as antigen (Calf thymus acetone powder 60 mg/ml, Pel-Freez, Rogers, AR). Litex agarose gel (FMC Bio products, Rockland, ME) and barbiturate buffer

(0.05 M, pH 8.6) were used for electrophoresis and 10 μl of ENA reagent applied to 20 μl of undiluted canine serum. The electrophoresis was run for 45 min using 120V and 44mA. Antibodies against ENA bind to the antigen and create a visible band of precipitation in the gel. The other method used was an ELISA anti ENA screening kit (Quanta Lite^{TM}Inova Diagnostics Inc. St. Louis, MO) which is composed of 6 purified autoantigens, all well-characterised in human diagnostics: SSA, SSB, RNP, Sm, Scl-70 and JO-1. The ELISA kit was modified for application with canine sera by using a rabbit anti-dog IgG peroxidase conjugate diluted 1:25000 (Sigma Chemical Co. St. Louis, MO), but otherwise following the procedure described for the kit which implies a serum dilution of 1:100. By the electrophoresis method 41 out of 141 patient sera (29%) were tested as positive wheras the result was 0/45 in the controls. Correspondingly the ELISA method gave 44 positives out of 129 sera (34.1%) and 1/45 in the controls. Positive reactions to all 6 specific ENA antigens could be detected among the positive anti ENA sera (unpublished).

One technique was established for detecting antibodies to chromatin (DNP) using ELISA kit with purified antigen (Novamed Ltd. Jerusalem, Israel) and applying the same adaptation for canine sera as for the ENA ELISA kit. As substrate for detecting antibodies to native DNA by IIF was utilized a protozoon, *Crithidia luciliae* (*Arden et al.* 1975). The crithidiae were cultivated in the laboratory and dispersed onto slides to be used in IIF. Like in the human variant a serum dilution of 1:10 was applied. The anti DNP test gave 53 positives out of 142 patient sera tested (37.3%) and 1/45 controls. The anti DNA method gave no positive reaction in any sera tested, which seems to correspond well with the findings of other investigators (*Hansson et al.* 1999, *Monier et al.* 1980, *Thoburn et al.* 1972).

Results

Figure 1 presents the picture of a typical paw of a Gordon setter with chronic symmetrical onychodystrophy showing small and stunted claws (A). B and C show the histopathological features with lichenoid infiltration of mononuclear cells at the dermo/epidermal junction, hydropic degeneration and apoptosis of keratinocytes in the basal layer and marked pigmentary incontinence.

Figure 2 presents a Gordon setter with marked black hair follicular dysplasia, and Fig. 3 presents the flank of another Gordon setter that had both black hair follicular dysplasia and symmetrical lupoid onychodystrophy (A). B and C show the histopathological sections. There were irregular clumping of pigment in hair shafts, malformed hairs in pilar canals and melanin in macrophages around the base of some follicles.

The grouping of the dogs according to their clinical symptoms, sex and age is presented in Table 1 and likewise the results of the testing for autoantibodies. As will be seen, no autoantibodies were detected in the controls. Seven of the diseased dogs were ANA positive and 2 had antibodies to ENA, both detected by the ELISA method.

The groups are too small to conclude anything about specific associations. The patient group as a whole is, however, significantly associated with positive ANA compared to the controls.

Discussion

The fact that the claw disease in the Gordon setter might be of lupoid character was the incitament for investigating ANA, anti ENA, anti DNA and anti DNP in serum from such dogs. *Scott et al.* (1995 a) found that 2 out of 12 dogs with symmetrical lupoid onychodystrophy were ANA positive. In our material 1 out of 7 was positive.When including dogs with other signs in addition to symmetrcal lupoid ony-

Table 1. Frequencies of antinuclear antibodies (ANA) and antibodies against extractable nuclear antigens (anti ENA) in healthy Gordon setters and in Gordon setters with symmetrical lupoid onychodystrophy and black hair follicular dysplasia, respectively. The anti ENA positive dogs were detected by the ELISA method.

Groups	n	years mean (range)	female/male	ANA* positive	anti ENA positive
Controls	21	4 (1-12)	11/10	0/21	0/21
Symmetrical lupoid onychodystrophy	7	7 (5-9)	2/5	1/7	0/7
Black hair follicular dysplasia	10	6 (1-11)	2/8	3/10	0/10
Symmetrical lupoid onychodystrophy and black hair foll. dyplasia	2	4.5 (3-6)	1/1	1/2	0/2
Symmetrical lupoid onychodystrophy and Mm long. dorsi pain	1	3	0/1	1/1	1/1
Black hair foll. dysplasia and Mm triceps brachii pain	1	3	0/1	1/1	1/1

*For ANA positivity: patients versus controls Fisher's exact test gives p= 0.009

chodystrophy, 3 out of 10 displayed ANA positivity.

Our suspicion that black hair follicular dysplasia and symmetrical lupoid onychodystrophy in the Gordon setter might somehow be connected, was the incitament for investigating the same parameters in serum from these dogs. We found that 3 out of 10 with black hair follicular dysplasia were ANA positive, while including 2 dogs with symmetrical lupoid onychodystrophy and 1 with muscle pain in addition to black hair follicular dysplasia, 5 out of 13 were ANA positive.

The present material shows an excess of male dogs in both disease groups. A slight overrepresentation of male dogs was also found in a greater material of Norwegian Gordon setters with symmetrical lupoid onychodystrophy (*Trotland, R.* pers. com. 1998) where the female/male distribution was 23/33. *Scott et al.* (1995 a) had both intact and spayed females and castrated males in their material. Black hair follicular dysplasia has previously been described in 1 Gordon setter (*Carlotti* 1990) and there is no large scale observation of sex ratio among Gordon setters with black hair follicular dysplasia in Norway. *Hargis et al.* (1991) reviewing the literature about black hair follicular dysplasia in dogs did not mention uneven sex ratio in their own and previous articles. They referred to the fact that the condition is heritable in mongrel dogs and also has an heritable basis in other breeds. The pups are normal at birth, but in the first few weeks of life abnormal coat is developed in black haired regions.

Black haired areas of the head and neck are less

severely affected. In black and red Doberman pinschers hair loss develops between 1 and 4 years of age, as in the Gordon setter, and hair loss is dorsally distributed on the lower back.

The question arises whether symmetrical lupoid onychodystrophy and black hair follicular dysplasia in the Gordon setter are signs or results of the same disease mechanism. The hair shedding occurs in younger dogs than does the claw shedding, and some dogs are shedding both black hairs and claws. After the hair shedding has occurred the hair coat never quite normalizes. There are ameliorating and worsening periods. After the claw shedding has occurred some dogs regain normal claws, while relapses and resulting small stunted claws for the rest of their lives are common. The fact that *Harvey* (1993) reported onychomalacia in" woolycoated" cavalier King Charles spaniels, and *Dunn et al.* (1995) diagnosed black hair follicular dysplasia in a 3-year-old female of the same breed with a poor fluffy hair coat, might indicate that these 2 conditions can occur together also in another breed of dogs.

Histopathological descriptions of these 2 phenomena in the Gordon setter are until now scarce. Until more thorough examinations are performed we would like to point out common features like degenerative and dysplastic changes in the epidermal basal cells and follicular cells, and pigmentary incontinence in the dermis. The difference observed in our material was that inflammatory reactions seen in symmetrical lupoid onychodystrophy were not present in black hair follicular dysplasia. A reason for that may be that histopathological specimens from the former group were taken in the acute phase, while specimens from the latter group were taken in the chronic phase.

The cutaneous affections described here have many features in common with the human skin disease alopecia areata which has a peak incidence in children and young adults. Alopecia areata has a genetic predisposition, is supposed to be an autoimmune disease mediated by T cells, and is associated to other autoimmune diseases like vitiligo, thyroid disease, Addison disease, diabetes mellitus, pernicious anemia, ulcerative colitis and SLE. The condition is characterized by a patchy, nonscarring depigmentation and shedding of hair. Ten to 44% of the patients have nail involvement as well, ranging from longitudinal ridging and thickening to friability and shedding (*Sahn* 1995, *Schwartz & Janniger* 1997).

Our findings also suggest that symmetrical lupoid onychodystrophy and black hair follicular dysplasia in the Gordon setter might be autoimmune diseases that are pathogenetically related , which might indicate a common genetic predisposition. The 2 diseases may together represent a canine equivalent of the human disease alopecia areata.

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Sammendrag

Antinucleære antistoffer (ANA) hos Gordon setter med symmetrisk lupoid onychodystrofi og svart hår follikel dysplasi.

Antinukleære antistoffer ble påvist i serum fra 3 av 10 Gordon settere med symmetrisk lupoid onychodystrofi, og i serum fra 5 av 13 Gordon settere med svart hår follikeldysplasi. To hunder hadde både symmetrisk lupoid onychodystrofi og svart hår follikeldysplasi, og en av disse var ANA positiv. Resultatene antyder at symmetrisk lupoid onychodystrofi og svart hår follikeldysplasi hos Gordon setter kan være autoimmune sykdommer med likhetstrekk i patogenesen. Dette kan indikere felles genetisk predisposisjon for de to sykdommene.

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