A Canine Case of Discoid Lupus Erythematosus with Circulating Autoantibody

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ABSTRACT. A nine-year-old Shetland sheepdog was diagnosed as discoid lupus erythematosus by clinical features, histopathologic findings, positive direct immunofluorescence, negative antinuclear antigen test and the absence of multisystemic diseases. The indirect immunofluorescence test of this patient dog with the salt split skin showed the deposition at the bottom of the cleft at basement membrane zone (BMZ). Western immunoblotting revealed the 120 kDa and the 85 kDa proteins targeted by the autoantibody. These proteins did not correspond with the known BMZ component. — KEY WORDS: canine, circulating autoantibody, discoid lupus erythematosus.

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Discoid lupus erythematosus (DLE) is a human and canine autoimmune skin disease characterized by facial erythematous plaque. DLE is differentiated from systemic lupus erythematosus (SLE) by the absence of multisystemic lesions and negative anti-nuclear antigen (ANA) test [13]. Cases of canine DLE have been reported since Griffin described the first case in 1979 [4, 9, 10–12, 14]. It is reported that collies, collie-cross breeds, Shetland sheepdogs, German shepherds and Siberian huskies are predisposed to canine DLE [11]. Canine DLE is a relatively benign disease and confined to skin and mucous membrane compared to SLE [11]. The common clinical symptom is nasal dermatitis and the condition is noted to become much worse in summertime. This group of dogs with DLE is identical to dogs that have previously been diagnosed as nasal solar dermatitis, or so-called "collie nose" [11]. The diagnosis of canine DLE is based on clinical features, dermatohistopathology, direct immunofluorescence (DIF) or lupus band test [13]. However, the detection of circulating autoantibody by indirect immunofluorescence (IIF) has not been reported in canine DLE. In this study, a canine case of DLE with positive DIF and IIF is reported.

A nine-year-old intact female Shetland sheepdog was presented to the Veterinary Medical Teaching Hospital of Gifu University with a two-month history of nasal erosion, crust formation, alopecia and depigmentation without pruritus (Fig. 1). The dog has not been medicated since the owner noticed the nasal lesion. The animal was found to be normal on hairy skin of head, extremities and trunk, and there was no hyperkeratosis on foot pad. The nose and nasal planum were depigmented and crusted (Fig. 1). A differential diagnosis of pemphigus erythematosus, Vogt-Koyanagi-Harada like syndrome, mycosis fungoides, DLE and SLE was made. The dog had a standard laboratory test for the differential diagnosis. Biopsy specimens were taken from the bridge of the nose. The biopsy specimen was cut into two pieces, the one fixed in 10% buffered formalin and the other immediately frozen and stored at -80°C.

The skin scraping, bacterial cultures and fungal cultures were negative. Complete blood analysis and serum biochemistry showed no abnormal values except high numbers of eosinophils (1,480/μl). One of the biopsy specimens was processed into paraffin-embedded sections and stained with hematoxylin-eosin. Histopathologic examination of the sections revealed the following epidermal changes: mild acanthosis, parakeratosis, hyperkeratosis, focal crust formation, focal mild spongiosis, leukocytic exocytosis and focal liquefaction degeneration of basal cells of epidermis and hair follicles (Fig. 2). The dermal changes were marked accumulations of mononuclear cells and plasma cells around mid-to-deep dermal blood vessels and apocrine glands.

ANA test using rat liver as substrate and patient serum (1:20) as the primary antibody was negative. DIF of skin using fluorescein isothiocyanate conjugated anti-dog IgG (Cappel, Durham, NC), IgM (The Binding site, Birmingham, UK) and C3 (The Binding Site, Birmingham, UK) revealed linear deposition of IgG and IgM at basement membrane zone (BMZ). Skin section of the patient showed no positive reaction when C3 was used as the antibody. The IIF (1:20) using the bovine tongue as substrate also revealed linear IgG class deposition at BMZ (data not shown). The salt split skin (SSS) was prepared from bovine tongue with the incubation in 1 M NaCl at 4°C for 96 hr. The epidermis and dermis separate at the level of lamina lucida in BMZ by this treatment [16]. IIF using SSS showed the linear IgG class deposition at the bottom of the cleft (Fig. 3). This result may imply that the canine patient serum with DLE has circulating autoantibodies and antigen which is targeted by the patient serum located below hemidesmosomes. Western immunoblotting of the patient serum (1:25) against cultured normal canine keratinocytes [5] was performed. The patient serum recognized the 120 kDa and 85 kDa proteins (Fig. 4). The definitive diagnosis of the patient was DLE from the crusted erythematous lesions confined to the nose, histopathologic findings of focal liquefaction degeneration of basal cells and the dermal accumulations of mononuclear cells, positive DIF, negative ANA and the absence of multisystemic diseases. The patient responded to predonisolone, tetracycline and niacinamide.

It has not been reported that any canine DLE patient has circulating autoantibodies by IIF. The patient in the present study revealed positive fluorescent deposition in both DIF and IIF at BMZ. The positive IIF might relate to the

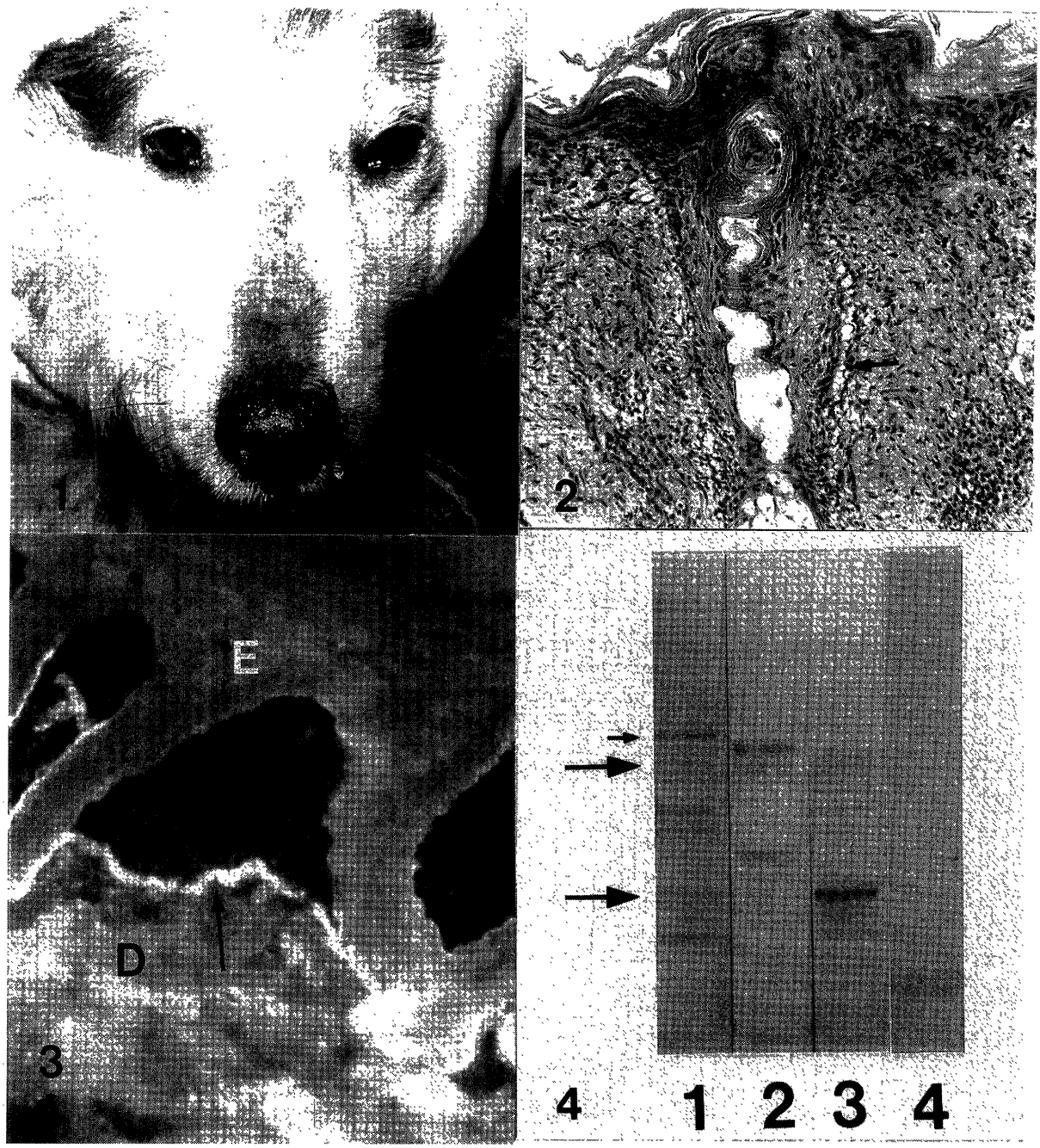

Fig. 1. Clinical appearance of a patient dog with discoid lupus erythematosus, showing depigmentation and crust formation confined to the bridge of the nose.

Fig. 2. The histopathology of the skin lesion on a patient with discoid lupus erythematosus, indicates the mononuclear cell infiltration in the dermis below the epidermis (lichenoid interface dermatitis) and the hydropic degeneration (arrow) at the basal keratinocytes.

Fig. 3. Fluorescent deposition (arrow) at the bottom of the cleft by indirect immunofluorescence in salt-split bovine tongue. (E): epidermis, (D): dermis

Fig. 4. Western immunoblotting of a patient serum against extracted proteins from cultured canine keratinocytes. On lane 1, the 145 kDa alpha chain of type VII collagen is seen (small arrow). On lane 2, laminin 5, anchoring filament proteins are labeled at the 135, 125 and 105 kDa. On lane 3, the patient serum recognizes the thick 120 and 85 kDa bands (arrows). Normal canine serum does not recognize specific proteins on lane 4.

presence of the circulating autoantibody in the DLE case. The localization of positive fluorescence by IIF in SSS was at the bottom of the cleft. Western blotting showed that the 120 and 85 kDa protein were recognized by a patient serum. Taken together, the patient serum may contain the autoantibodies against BMZ macromolecules which locate below the lamina lucida. However, it is not reported that the specific proteins are targeted by circulating autoantibody besides nuclear proteins in a DLE patient. BMZ proteins targeted by the autoantibodies in human autoimmune skin diseases are type VII collagen in epidermolysis bullosa acquisita (EBA) [15], bullous pemphigoid (BP) antigen in BP [8], several antigens in paraneoplastic pemphigus [1] and herpes gestation [3]. The patients with epidermolysis bullosa acquisita have the autoantibodies against the NC-1 domain of type VII collagen which localizes at the bottom of SSS, and the patient serum recognizes the 290 and 145 kDa proteins in keratinocytes [15]. Human BP patients have autoantibodies to the 230 and 180 kDa BP antigen which locate at hemidesmosomes [8]. A dog patient with BP shows the 180 kDa BP antigen [6]. When SSS is used as the substrate of IIF with a BP patient serum, the roof of the cleft exhibits positive fluorescence [6, 8]. Human patients with herpes gestation have the autoantibody against the 180 kDa BP antigen which locates at the roof of SSS [3]. Paraneoplastic pemphigus is a human autoimmune blistering disease in patients with lymphoreticular malignant tumor [1]. The patients of this disease have the autoantibodies against desmoplakin I and II (250 and 190 kDa) and 230 kDa BP antigen [1]. Desmoplakins exist at the intercellular space of epidermal cells. Recently reported new human autoimmune blistering skin disease relates with the novel 105 kDa protein which localizes at the bottom of SSS [2]. When we compare the autoantigens targeted by these autoimmune skin diseases with the autoantigen in our DLE case from the stand point of the molecular size and localization, the localization of type VII collagen and the 105 kDa protein corresponded with that of the DLE patient, but the molecular sizes of the targeted protein were not identical. The localization of BP antigen in BP and herpes gestation are different from that of the DLE patient. Therefore, the proteins targeted by the autoantibodies of the DLE patient cannot be identified as the antigens targeted by the reported autoimmune diseases. However, the alteration of BMZ macromolecules, especially in type IV and type VII collagen, is reported in human DLE patients [7]. Future studies will need to focus on the BMZ proteins to provide further insights into the mechanisms of canine autoimmune skin diseases.

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