Immune Mediated Skin Lesions in a Dog with Autoimmune Hemolytic Anemia

Takashi HASEGAWA, Kiyomi MATSUOKA, Kenichiro ONO¹⁾, Toshihiro WATARI, Ryo GOITSUKA, Ryohei NISHIMURA²⁾, Hiroyuki NAKAYAMA³⁾, Naoaki GOTO³⁾, and Atsuhiko HASEGAWA

Department of Veterinary Internal Medicine, ¹⁾Department of Veterinary Clinical Pathobiology, ²⁾Department of Veterinary Surgery and ³⁾Department of Veterinary Pathology, Faculty of Agriculture, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113, Japan

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ABSTRACT. A canine case of Coombs' test positive and antinuclear antibody-negative hemolytic anemia was examined because of the development of skin lesions after 18 months treatment with prednisolone. Histopathological examination of biopsy specimens obtained from skin and oral mucosa revealed the acantholysis, edematous lesions of the stratum basale and mononuclear cell accumulation in the dermis. Deposits of immunoglobulin G and complement factor 3 were detected at the intercellular and dermoepidermal junction by the direct immunofluorescent test. From these results, the case was considered to be an autoimmune disease caused by distinct antibodies against different organs.—KEY WORDS: dog, hemolytic anemia, skin lesion.

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Immune mediated skin diseases are classified into two groups, bullous autoimmune skin disease including pemphigus and pemphigoid [7, 8, 13] and probable or possible immune mediated non-bullous skin diseases such as discoid lupus erythematosus and panniculitis [13]. In the former, the organ-specific autoantibody against the cell surface antigen on the keratinocytes and/or basement membrane [10, 18] was detected, and in the latter, organ-specific antibody was not observed. However, the complicated cases were reported especially in the pemphigus erythematosus which is considered to be a hybrid between pemphigus foliaceus and systemic lupus erythematosus (SLE) [12].

On the other hand, many cases of canine autoimmune hemolytic anemia (AIHA) were reported since 1954, suggesting that AIHA might be a relatively common cause of anemia in the dogs [3, 7, 9, 11, 14, 15, 16, 19]. However, there is no report on the AIHA associated with immune mediated skin diseases and/or immune mediated skin diseases associated with AIHA, except

SLE. This report deals with immune mediated skin lesions detected in a canine AIHA.

MATERIALS AND METHODS

Case: A 4 year-old intact female long-haired dachshund weighing 4.6 kg was presented to the Veterinary Hospital, Faculty of Agriculture, The University of Tokyo, with the complaints of weakness, vomiting and anorexia. At the first admission, no abnormalities were detected except pale mucous membrane and systolic murmur on physical examination. The laboratory findings are given in Table 1. Abnormal findings included a regenerative anemia (RBC: $1.16 \times 10^6/\mu\text{l}$, Hb: 4 g/dl, PCV: 10%, Reticulocyte: 6%), mild leukocytosis, and increases of serum alkaline phosphatase (ALP) and aspartate aminotransferase (AST) levels. The osmotic fragility of erythrocytes increased as compared to clinically healthy dogs. The direct and the indirect Coombs' tests [4, 17] revealed

Table 1. Laboratory findings on the first admission

PCV (%)	10	T.P. (g/dl)	7.0	Osmotic fragility	
RBC ($\times 10^6/\mu\text{l}$)	1.16			50% hemolysis (%NaCl)	0.53
Hb (g/dl)	4	BUN (mg/dl)	18.9		
WBC ($/\mu\text{l}$)	15800	T. Bilirubin (mg/dl)	0.3	Coombs' test	
Band (%)	5			Direct	
Seg (%)	88	ALP (U/l)	269	Anti-IgG	+
Lympho (%)	6	ALT (U/l)	40	Anti-IgM	-
Eosino (%)	1	AST (U/l)	131	Anti-C3	-
Platelet ($\times 10^3/\mu\text{l}$)	239	LDH (U/l)	112	Indirect	+
Reticulocyte (%)	6			Antinuclear antibody	-

Fig. 1. Clinical course.

positive reactions, however, no antinuclear antibody (ANA) was detected. As shown in Fig.1, the dog was treated with prednisolone (1 mg/kg/day). The hematologic abnormalities returned to normal after 2 weeks, and then drug doses were gradually reduced. The patient was in good condition for seven months without treatment. After that, however, it was suddenly attacked by severe anemia (RBC: $0.97 \times 10^6/\mu\text{l}$, PCV: 5%).

At 18 months after first medication, when the anemia was almost recovered, edematous and erosive skin lesions in the footpad, tongue and lip were developed (Fig. 2).

Histopathological examination: Skin and oral mucous membrane were biopsied for histopathological examination. Biopsy specimens were fixed in 10% neutral buffered formalin and embedded in paraffin. Section 4 μm thick was made by a routine procedure and stained with hematoxylin and eosin (HE).

Immunological examination: Direct and indirect immunofluorescent tests were performed to detect immunoglobulin (Ig)G and complement factor 3 (C3) in biopsy specimens. For direct immunofluorescent test, biopsy specimens from the patient were frozen to -80°C in cold hexane. These were

Fig. 2. Severe edematous and erosive lesions in the tongue.

embedded in Tissue-Tek OCT Compound (Lab-Tek Products, Naperville, Illinois, U. S. A.), cut with a cryostat microtome at 5 to 6 μm , dried well, and fixed in cold acetone. Sections were washed in phosphate-buffered saline (PBS, pH 7.4) and stained with fluorescein isothiocyanate (FITC)-conjugated goat anti dog IgG and C3 (Cappel Lab, U. S. A.). For indirect immunofluorescent test, frozen sections from the footpad, tongue and lip of a healthy dog were used. Sections were overlaid with the patient serum, incubated at

37°C for 30 minutes, washed with PBS, and stained with FITC-conjugated goat anti dog IgG. All specimens were examined under fluorescent microscopy.

RESULTS

Histopathological examination: The lesions in the tongue were characterized by marked acantholysis, severe mononuclear cell infiltration in the dermis and stratum spinosum (Fig. 3a). A suprabasilar intraepithelial microvesicle containing degenerated acanthocytes, lymphocytes and plasma cells was seen (Fig. 3b). In the tongue lesions, muscular atrophy and interstitial infiltration of lymphocytes were detected. Labial lesions included edema of the basal cell layer, spinocellular vacuolation, and mild infiltration of lymphocytes in the superficial dermis (Fig. 4). The mild edema of basal cell layer, degeneration of sebaceous gland and infiltration of mononuclear cells in the dermis, especially periglandular, periductular, and superficial infiltration of the dermis were seen in the footpad. Moreover, dermal lesions included atrophy of connective tissue.

Immunological examination: By the direct immunofluorescent test, IgG was demonstrated in the epidermal intercellular spaces

Fig. 3. a) An acantholytic spinocellular layer and mononuclear cell infiltration in the lamina propria and stratum spinosum in the tongue. Bar=100 μm .
b) A suprabasilar intraepithelial microvesicle containing mononuclear cells admixed with acantholytic cells. Bar=25 μm .

Fig. 4. Edematous lesion of the basal cell layer and infiltration of lymphocytes in the superficial dermis in the lip. Bar=25 μ m.

in stratum spinosum of the tongue, lip and footpad (Table 2, Fig. 5a). Moreover, IgG was detected at the dermoepidermal junction of the tongue and footpad (Table 2, Fig. 5b). The C3 deposits were also found in the epidermal intracellular spaces in the stratum spinosum of the tongue (Table 2, Fig. 5c). By the indirect immunofluorescent test with patient serum, no antibodies to spinocell and dermoepidermal junction were detected (Table 2).

DISCUSSION

In this AIHA case, edematous and erosive lesions were developed in the tongue, lip and footpad. Histopathological changes observed in the present case were characterized by the basal layer, and mononuclear cell accumulation in the superficial dermis with

various degree. Additionally, IgG and C3 deposits were confirmed in intercellular epidermal area and dermoepidermal junction by the direct immunofluorescent test, which indicated the presence of the autoantibodies against spinocells and dermoepidermal junction. These results suggested that the skin lesion observed in the case was considered as pemphigus erythematosus [2, 7, 13].

Halliwell *et al.* [7, 8] suggested that circulating autoantibodies could rarely be observed in canine skin specimens by the indirect method. In this case, no immunofluorescence was also detected by indirect method. The circulating autoantibodies were considered to have a minor role in canine autoimmune skin diseases [7, 8].

Halliwell [8, 9] demonstrated that an important diagnostic criteria for canine SLE was the presence of nonorgan-specific antibody such as ANA. He also proposed that the probable SLE could be diagnosed with two major clinical signs such as polyarthritis, AIHA, thrombocytopenia, and glomerulonephritis without detection of the antibody. On the contrary, it has been widely accepted that serum ANA should be detectable in human SLE cases [1]. When skin lesions were developed in this AIHA case, no other clinical signs associated with SLE were observed and the test for ANA was negative. From these results, the case could not be diagnosed as SLE. Since 3 organ specific antibodies against erythrocyte

Table 2. Immunofluorescence for skin biopsy specimens

Specimen	Lesion	Direct		Indirect
		IgG	C3	
Footpad	Epidermal intercellular	+	+	—
	Dermoepidermal junction	+	—	—
Lip	Epidermal intercellular	+	—	—
	Dermoepidermal junction	—	—	—
Tongue	Epidermal intercellular	+	+	—
	Dermoepidermal junction	+	—	—

+: Positive for deposits, —: Negative for deposits.

Fig. 5. Direct immunofluorescent test.

a) IgG was in the intercellular spaces in the tongue. Bar=5 μ m.

b) IgG deposition at the basement membrane zone in the footpad. Bar=5 μ m.

c) C3 was in the intercellular spaces in the tongue. Bar=5 μ m.

cell membrane, spinocells, and dermoepidermal junction were detected, the present case was considered to be similar to the overlap disease showing several organ-specific autoantibodies reported in man [5, 6, 12].

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要 約

自己免疫性溶血性貧血の犬に認められた免疫介在性の皮膚病変：長谷川貴史・松岡清美・小野憲一郎¹⁾・亘敏広・後飯塚 僚・西村亮平²⁾・中山裕之³⁾・後藤直彰³⁾・長谷川篤彦(東京大学農学部獣医内科学教室，¹⁾獣医臨床病理学教室，²⁾獣医外科学教室，³⁾獣医病理学教室)——抗グロブリン試験陽性，抗核抗体陰性でプレドニソロン治療18ヵ月後に皮膚病変を発現した自己免疫性溶血性貧血の犬を精査した。皮膚および舌の病理組織学的検査では棘融解，基底膜の浮腫および真皮に単核球の浸潤が認められた。また，蛍光抗体直接法では細胞間と真皮表皮接合部にIgGとC3の沈着が観察され，紅斑性天疱瘡のそれと類似するものであった。以上のように本例においては複数の臓器に対する自己抗体が産出されていることが確認された。