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Discoid lupus erythematosus (DLE) in a juvenile Alaskan Malamute dog

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Abstract : Discoid lupus erythematosus (DLE) is an immune-mediated skin disease which requires histopathology and immunohistopathology in both dogs and humans. A 10-month-old, intact female Alaskan Malamute presented for depigmentation, swelling, alopecia, erythema, and crusting on the bridge of the nose and the nasal planum. Cytological examination of nasal lesions revealed numerous cocci and neutrophils. Histopathological features included of infiltration of mononuclear cells at the dermoepidermal junction. Direct immunofluorescence tests and immunohistochemistry exhibited positive IgG, IgM, IgA, CD3, CD18, and CD79a on the epidermal basement membranes and around adnexal glands. This case indicates both T cells and B cells are related to mechanism of canine DLE. This case report describes advanced diagnostic tests and clinical outcome with immune suppressive therapy in a rare juvenile canine DLE case.

Keywords : discoid lupus erythematosus, dog, immunohistochemistry, immunohistopathology

Introduction

Discoid lupus erythematosus (DLE) is the second most common immune-mediated dermatitis in dogs and is a relatively benign skin disease without systemic involvement [4, 9]. Clinical signs of initial presentation include depigmentation, erythema, scaling, erosion, ulceration, and crusting nasal planum and bridge of the nose. The diagnosis of the DLE requires a compatible medical history, appropriate signalment, routine diagnostic skin tests, and a histopathologic examination with characteristic findings. Definitive diagnosis typically requires confirmation with immunofluorescence and immunoperoxidase methods [8]. In humans, it has been demonstrated that the lymphocytes infiltrating skin lesions of DLE are predominantly T cells, on the other hand, B lymphocytes are dominant in the dogs [9].

This case report describes a diagnosis of canine DLE by clinical features, histopathology, and immunohistopathological examination. In addition, clinical outcome following immunosuppressive therapy is also described.

Case report

A 10-month-old, intact female Alaskan Malamute presented to our teaching hospital for depigmentation, alopecia, erythema and muzzle swelling with a 2-month history. The dog lived in an outdoor rooftop environment. Physical examination revealed swelling, depigmentation, erythema, alopecia, and mild crusting on the bridge of the nose and the nasal planum (Figs. 1A and B). The periocular regions were swollen bilaterally without ocular discharge. Oral dexamethasone was intermittently administered by referring veterinarian. Only a mild leukocytosis was shown in complete blood count, and the panel of serum biochemistry was within normal limits. No significant findings were revealed in nasal radiography. Muzzle cytology with tape preparation showed some neutrophils and cocci. Topical mupirocin (Bactroban ointment; Hanall Pharmaceutical, Korea)

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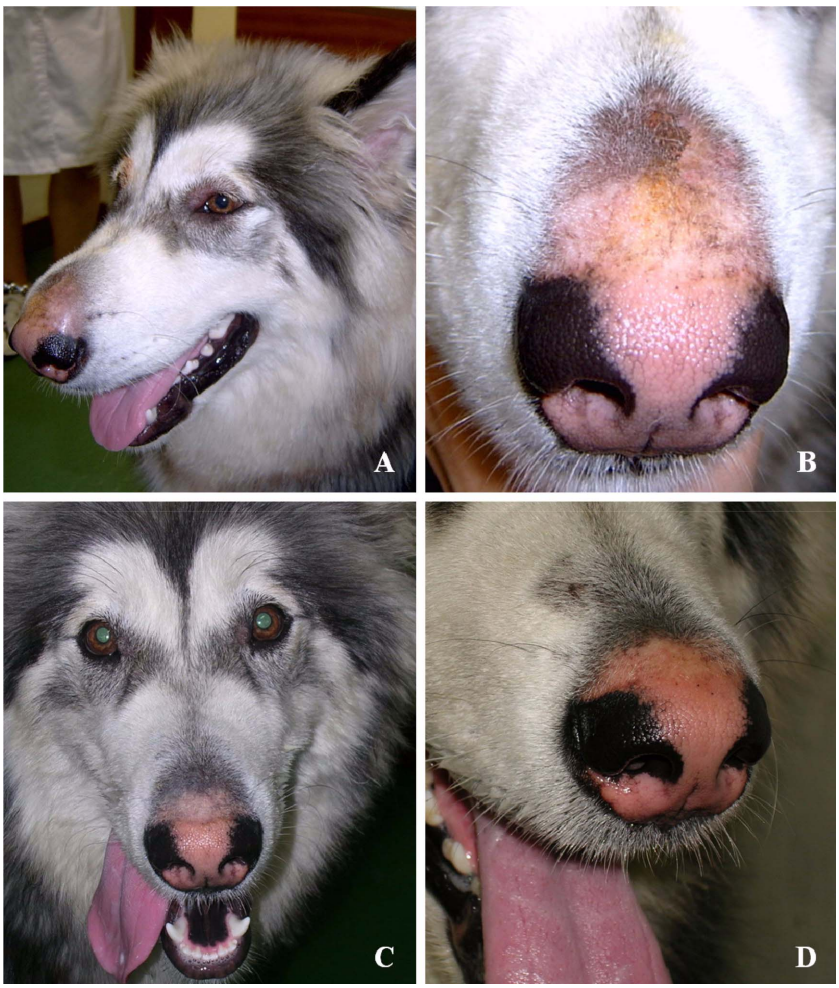

Fig. 1. An Alaskan Malamute with discoid lupus erythematosus. (A): Swelling of the periocular region and muzzle. (B): Magnified view of the muzzle and nasal planum, with depigmentation, alopecia, erythema, and crust. (C) and (D): Complete resolution of lesions after 4 weeks of corticosteroid therapy.

and systemic oral cephalexin (Cefulen, 30 mg/kg body weight; Newgenpharm, Korea) were administered at twice daily dosing to rule out bacterial infection. This antibiotic therapy was ineffective. Fungal cultures were negative.

Skin biopsies were taken from muzzle for histopathological examination. Histopathology revealed sharp demarcations between the epidermis and dermis, and the dermoepidermal junction was partially obscured by interface inflammation. A lymphocyte-rich infiltration resulted in interface dermatitis along the dermoepidermal junction and adnexal glands. There were prominent basal cell degenerations and this resulted in an indistinct dermal-epidermal junction (Figs. 2A and B). We considered DLE and some other autoimmune skin diseases, which have similar histopathological features such as SLE.

Immunofluorescence and immunohistochemistry were performed to confirm the presumptive diagnosis. Immunofluorescence tests were carried out with goat anti-dog IgG monoclonal antibody (1 : 100; Bethyl Laboratories, USA), rabbit anti-IgM (1 : 100; Bethyl Laboratories, USA), C3 (1 : 200; Bethyl Laboratories, USA), and mouse anti-human CD79a (1 : 200; Dako-Cytomation, Denmark) in PBS containing 1% Triton X-100. For immunohistochemistry, primary antibodies were used in this study: rat anti- human CD3 (1 : 1,000; Serotec, Germany), mouse anti-canine CD18 (1 : 500;

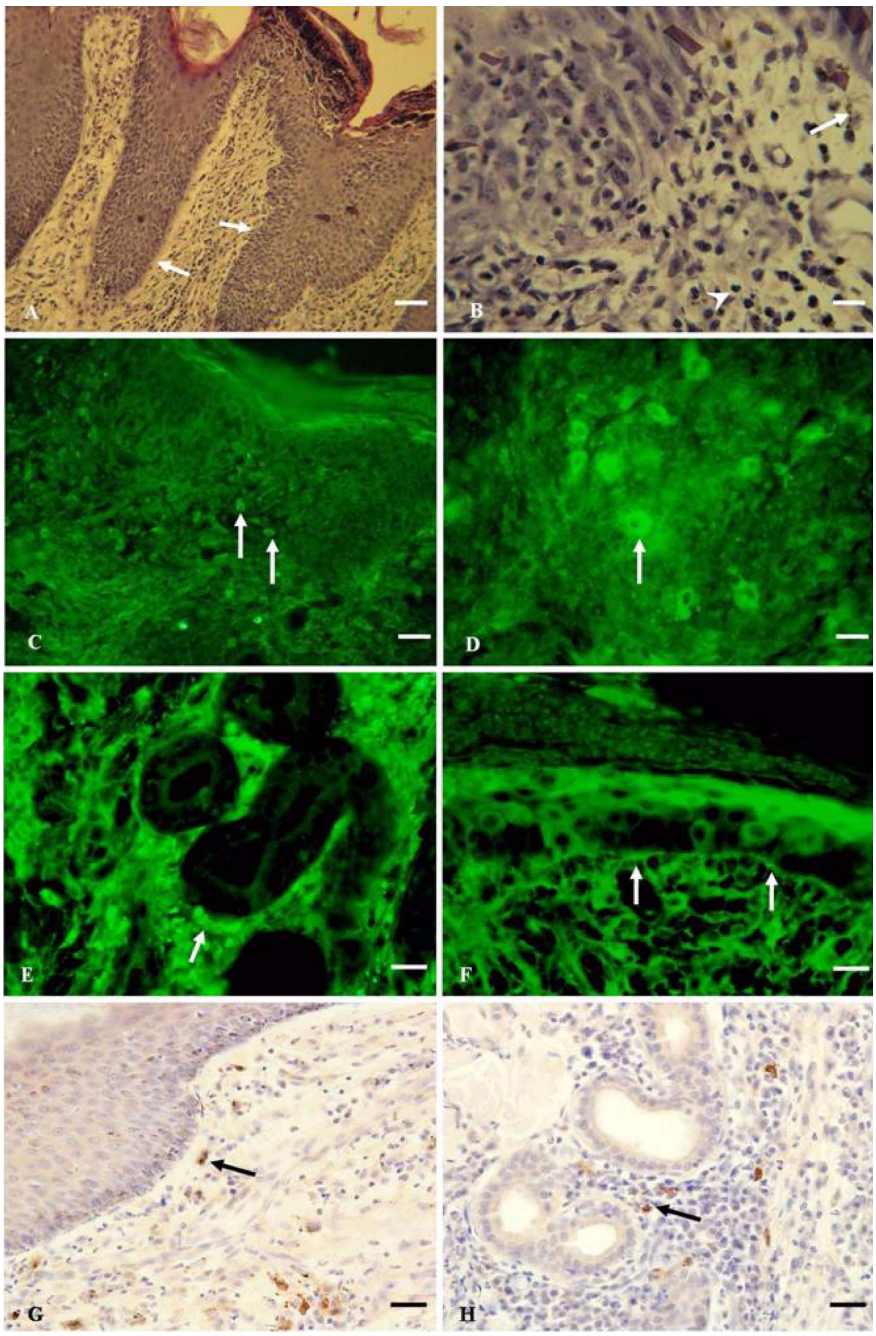

Fig. 2. Photomicrographs of histopathology in canine discoid lupus erythematosus case (A and B). Immunofluorescence stains and immunohistochemistry for demonstration of immunoglobins (C-H). (A): Lichenoid interface dermatitis with thickening of the basement membrane (×200, Hematoxyline and eosin stain), (B): Higher magnification of dermoepidermal junction seen in the A. Note pigmentary incontinence (arrow), keratinocytes apoptosis (arrowhead), and vacuolation of the dermoepidermal region (×400, Hematoxylin and eosin stain). Immunofluorescence stains for IgM (C, arrow), IgA (D, arrow), and IgG (F, arrows) show fine depositions within the dermoepidermal junction. IgG (E, arrows) show deposition around adnexal glands. (G) and (H): The IgA-positive cells detected in dermoepidermal junction (G, arrow) and around sweat glands (H, arrow) by immunohistochemistry. Scale bar, 70 μm in A, C, E, and G; 35 μm in B, D, F, and H.

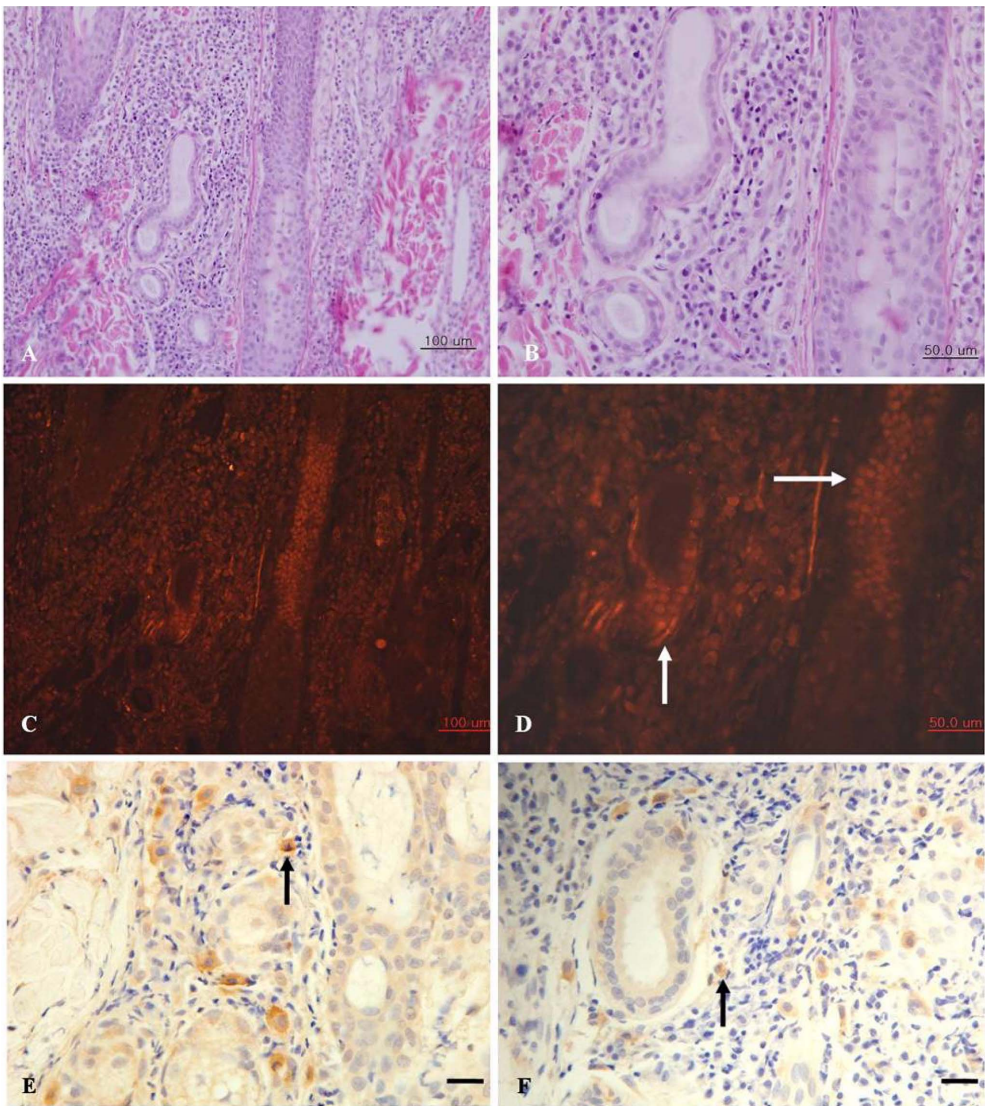

Fig. 3. Skin biopsy on the muzzle of a dog with discoid lupus erythematosus (DLE). Immunofluorescence stains and immunohistochemistry demonstrate both T cells and B cells within canine DLE lesions. (A): The epithelial infiltrate is composed of lymphocytes and plasma cells in the the follicular infundibulum and around sweat glands (×200, Hematoxylin and eosin stain). (B): Higher magnification of A (×400, Hematoxylin and eosin stain). (C) and (D): Immunofluorescence for CD79a reveals deposition in the same region as A and B (arrows). (E): CD3 immunohistochemistry revealed positive reactions around sebaceous glands in (arrows). (F): CD18-positive cells were detected around sweat gland regions by immunohistochemistry (arrows).

Serotec, Germany), and rabbit-anti dog IgA (ICN Biochemical, USA).

Direct immunofluorescence staining performed on the paraffin-embedded sections revealed *in situ* IgM, and IgA deposition in the epidermal basement membrane and superficial dermis, IgG deposition was shown around adnexal glands as well as basement membrane (Figs. 2C-F). IgA was also confirmed by immunohistochemistry in basement membrane and around sweat glands (Figs. 2G and H). C3b deposition in superficial basement membrane was not observed. Immunohistochemistry confirmed the presence of CD3-positive T lymphocytes and CD18-positive histiocytic cells in the lesions (Fig. 3F). CD3- and CD18-positive cells were

also present in large numbers around adnexal glands. Direct immunofluorescence stains revealed CD79a in the follicular infundibulum epithelium and around sweat glands (Figs. 3C and D). Therefore, we confirmed the diagnosis of DLE based on the histopathology, direct immunofluorescence staining, and immunohistopathology.

A topical betamethasone and gentamicin complex ointment (Gentizone; Hutecs, Korea) and oral cephalexin (30 mg/kg body weight) were administered twice daily for 2 weeks. The lesions gradually improved, but clinical relapse occurred after the discontinuation of therapy. Therefore, prednisolone (Prednisolone; Korea Pharma, Korea) was orally administered once daily at a 2.2 mg/kg. The muzzle and periocular lesions were responsive to treatment and completely resolved after 4 weeks of therapy (Figs. 1A and B).

Discussion

Although no age predilection has been reported in canine DLE [9], to authors' knowledge, there are no case reports in canine DLE that is related to juvenile Alaskan Malamute dog. In this case, the dog was 10-month old with a 2-month history.

The patient in this case lived in a rooftop outdoor environment, and clinical signs appeared during the summer season. This seasonal worsening is a characteristic of DLE as clinical signs are exacerbated by sun exposure in about 50% of cases [6, 9].

DLE diagnosis requires both clinical evaluations and the demonstration of typical histopathological features [5, 7, 11]. Histopathological features in SLE are frequently indistinguishable from those in DLE, and therefore anti-nuclear antibody (ANA) tests and further evaluations are frequently required to rule out SLE [7]. ANA test was not performed in this case because of previous steroid administration by the referring veterinarian. Dog with SLE typically present with clinical systemic illness, such as anemia, thrombocytopenia, proteinuria, fever of unknown origin, polyarthritis, neutropenia, and central nervous system disease [2, 3, 7]. But this patient showed no systemic signs and the blood work and urinalysis revealed no abnormalities.

Multiple CD3- and CD18-positive cells, IgA-positive cells, and IgG-positive cells were detected in the follicular infundibulum, adnexal glands, and sweat glands. It was not typical characteristic of the DLE in dogs. A previous study has reported that sebaceous gland and hair follicle level as well as basement membrane zone were also included in immunofluorescence test [1]. However, it was exfoliative cutaneous lupus erythematosus in German short-haired pointers.

The presence of CD3-positive T lymphocytes and CD18-positive histiocytic cells in the epidermal, follicular and sebaceous gland basement membranes suggest a major histocompatibility complex-restricted-cell mediated immunological reaction to antigens shared by these regions [1].

CD79a-positive B lymphocytes were highly distributed in this case. Therefore, humoral immune responses may be occurred in this lesions, which supported the previous theory which demonstrated plasma cell dominant character in canine DLE pathogenesis.

In this case, glucocorticoids were used due to relatively immediate clinical effects and lack of expense. We slowly tapered the prednisolone dosage to avoid clinical side effects and advise the client to monitor any recurrence of lesions.

In conclusion, we examined DLE in the dog with a panel of markers for immunological mechanisms antigens that included antibodies CD3, CD18, CD79a, IgG, IgM, and IgA. Further studies are required to investigate correct pathogenesis of canine DLE.

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