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<Case Report>

Alopecia areata in a spayed pug: clinical and immunohistochemical findings

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Abstract : A two-year-old spayed female pug presented with symmetrical hyperpigmented alopecic lesions on her axillary and inguinal regions. There were no remarkable findings in dermatologic examinations and hormonal assays. Histological examination of biopsied tissues revealed prominent lymphocytic perifolliculitis along with shrunk hair follicles. Immunohistochemistry for CD3, CD79a, CD4, and CD8 showed a positive stain for CD4 antigen around hair bulbs, suggesting CD4 positive T lymphocyte infiltration. This case suggests the possibility that CD4 T lymphocyte-mediated inflammatory reaction could be a main mechanism in canine alopecia areata. Additional studies are warranted to investigate the immunological mechanism in canine species.

Keywords : alopecia areata, autoimmune disease, T lymphocyte

Canine alopecia areata (AA) is an uncommon or infrequently recognized disease believed to be similar to the human disease [7]. The disease is predominantly caused by a T-cell-mediated autoimmune etiology [9]. Clinical signs include asymptomatic, noninflammatory, and well-circumscribed alopecia, and the lesion is often symmetric and hyperpigmented [4, 9]. A definitive diagnosis can be made based on the insidious progress of asymptomatic, well-circumscribed alopecia and histopathologic characteristics including miniaturized hair follicles and lymphocytic perifolliculitis. The prognosis is usually good, with spontaneous recovery in 60% of cases. If recovery is not seen in a month, treatment with intralesional, topical, or systemic immunosuppressive therapy is often effective [2, 6].

In this case report, we describe clinical and immunohistochemical findings of AA in a dog that was diagnosed by clinical features, dermatological, histopathologic, and immunohistochemical examinations.

A two-year-old spayed female pug presented with a one-year history of symmetric and hyperpigmented alopecia. On physical examination, the alopecia was found on the bilateral axillary, inguinal, and median thigh regions (Fig. 1).

There was no abnormality in the laboratory examinations, which included a complete blood count and serum biochemistry profiles. Dermatologic examinations including skin scraping, cytology, and fungal culture on the alopecic region also presented no remarkable finding. Nor did hormonal assays for adrenal and thyroidal functions show any abnormalities. Skin biopsies were performed on the right axillary, left inguinal and right median thigh regions, and the tissues were fixed in 4% neutralized formalin. The fixed tissues were then processed to make paraffin-embedded tissue sections. The sections were examined with hematoxylin and eosin staining under a light microscope. Histopathologic examination showed a mild proliferation of the epidermis with a dense keratin layer, hyperpigmentation, and mild lymphocytic infiltration in the dermis (Fig. 2). Lymphocytic infiltrations were also found

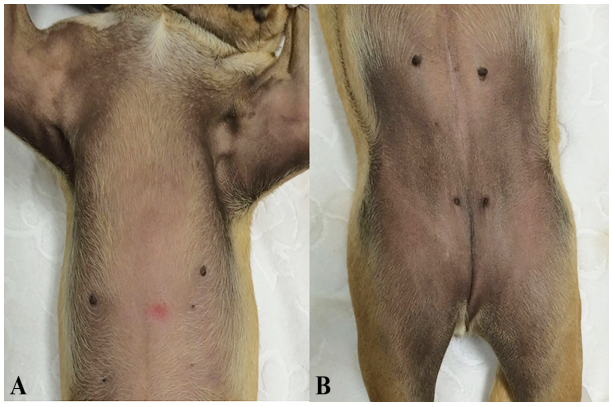

Fig. 1. Clinical features presented symmetric alopecia and hyperpigmentation on the axilla (A), inguinal region, and median thigh (B) in a pug dog.

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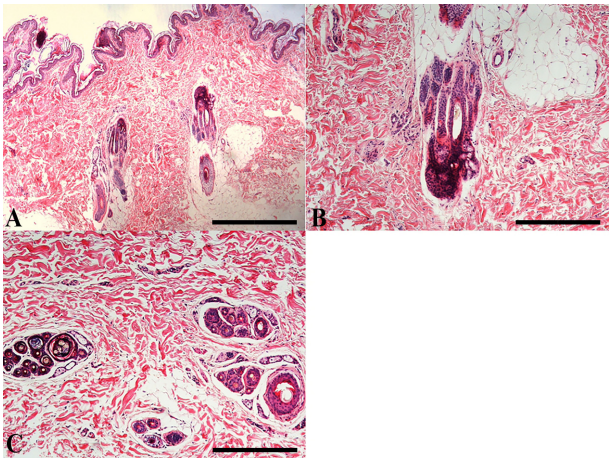

Fig. 2. Histopathological features of alopecic lesion representing (A) mild proliferation of epidermis with a dense keratin layer, hyperpigmentation, and mild lymphocytic infiltration in dermis, (B) lymphocytic infiltration in the hair roots, and (C) a miniaturized hair follicle. H&E stain. Scale bars = 500 μm (A), 200 μm (B and C).

around the sebaceous glands and hair bulbs. Most of the hair follicles were found in an anagen phase; however, some of them were shrunk with mild lymphocytic infiltration.

Immunohistochemistry (IHC) examinations were performed to classify the subtype of infiltrated lymphocytes in the lesion. Monoclonal antibodies against CD79a for the B lymphocyte (Santa Cruz Biotechnology, USA), CD3 for the T lymphocyte (Abcam, UK), CD4 for the helper T lymphocyte (Abcam), and CD8 for the cytotoxic T lymphocyte (Abcam) were selected. The sections were counterstained with Mayer's hematoxylin and were dehydrated and mounted. IHC showed that most of the infiltrated lymphocytes were positive to CD3 and CD4 and were negative to CD79a and CD8 (Fig. 3), indicating that they were helper T lymphocytes. With all of this taken together, the dog was finally diagnosed with helper T lymphocyte-mediated AA. Further medical treatments were not made due to the owner's unexpected return to his country.

AA has a complex pathogenesis in both humans and dogs [1]. It is believed that the disease is induced by an immune-mediated mechanism that can interrupt the normal hair cycle [1]. Infiltration of T lymphocytes affecting anagen hair follicles subsequently leads to hair follicle miniaturization and defective hair shaft formation, resulting in clinically visible hair loss [9]. Due to the process of miniaturization, the affected hair bulbs are located in the upper dermis in chronic stages of the disease [5, 8]. Based on physical and dermatologic examinations, we excluded demodicosis, dermatophytosis, bacterial pyoderma, mural folliculitis, and hormonal diseases. Follicular dysplasia and pattern alopecia were also excluded by histopathology. In this case, the histopathologic results showed pathognomonic changes of the hair follicles with the infiltration of helper T lymphocytes and miniaturization of anagen hair follicles in the upper dermis. This most important histopathologic features and exclusion of other diseases allow the definitive diagnosis of AA in the dog.

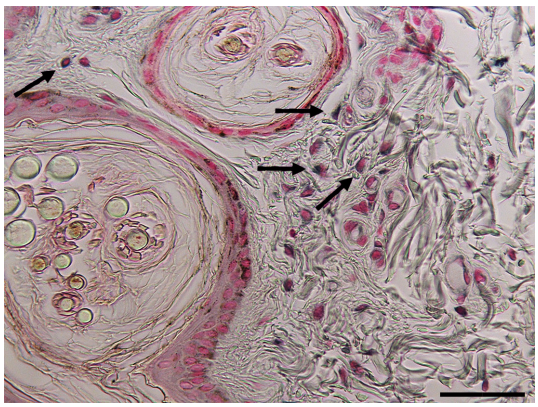

Fig. 3. Immunohistochemistry of the inflamed hair follicles representing CD4 positive lymphocyte infiltration (arrows) around the hair follicle. Mayer's hematoxylin counterstain. Scale bar = 50 μm.

Canine AA is believed to be a good exemplar of human AA with respect to the aspects of histopathologic and immunologic patterns that include peribulbar CD4 and CD8 T lymphocyte infiltration and T lymphocytes and IgG depositions around anagen hair follicles [9]. However, all of the infiltrated lymphocytes in the alopecic areas in this case were positive to CD4, but none of the cells were positive to CD8, while the positive control for CD8 showed appropriate expression to the CD8 antigen, indicating that the alopecia was provoked by the CD4 lymphocyte-induced perifollicular inflammation in this case. Previous studies have suggested that direct modulation of hair loss is achieved by the CD8 T lymphocyte in AA, while the CD4 lymphocyte promotes the onset of AA in humans and rodents by activating the host's immune system [1, 3]. We were not able to answer exactly the question why negative to CD8 antigen in IHC on this case. Infiltration of CD4 lymphocyte alone in alopecic region shown in this case might be related to different roles of CD4 lymphocyte as contrast with human AA. It is possible that CD4 lymphocyte could have roles both onset of disease and modulation of hair loss in canine AA. Further studies of AA on immunological mechanism are needed to clarify its characteristics in dogs compared to other species.

In conclusion, this case suggests the possibility that CD4 T lymphocyte-mediated inflammatory reaction around hair bulb region could be a main mechanism in canine AA. To our knowledge, this is the first case of canine AA in our country.

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