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A case of feline extramedullary plasma cell tumor with T cell infiltration

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Abstract

A 7-year-old castrated male Persian cat presented with a cutaneous mass and an increase in serum amyloid A concentration. Fine needle aspirates of the mass indicated lymphoma, which was also the top differential diagnosis on histopathologic examinations. Immunohistochemically, neoplastic cells tested negative for anti-CD3, PAX5, CD20, and c-Kit, but positive for MUM1, CD79α, and CD138, suggesting extramedullary plasmacytoma. There were tumor-infiltrating non-neoplastic $CD3^+$ T and $PAX5^+$ B cells. Practitioners should be aware of feline plasmacytoma characterized by lymphoma-like cytologic and histologic features. The present study is valuable in providing the first clinical evidence that proves the immunogenicity of feline plasmacytoma.

Keywords: cats; tumor infiltrating lymphocyte; synthetic immunogens; plasmacytoma

Plasmacytoma (PCT)–a round cell tumor originating from differentiated B cells–is a myeloma-related disorder (MRD) that is anatomically classified as multiple myeloma (MM) and extramedullary plasmacytoma (EMP) [1,2]. The incidence of feline EMP has not been well-documented but is considered extremely rare [3,4], given that MRD accounts for 0.003 to 0.1% of all feline malignancies [5]. Feline cutaneous EMP is often benign, which could be cured by complete excision, showing rare recurrence [4]. Cytologic and histopathologic examinations usually lead to a definite diagnosis of EMP based on the morphologic features of plasma cells. Immunohistochemistry (IHC) aids the diagnosis by using staining positivity for MUM1, a marker defining plasma cell differentiation [6]. In this study, we report a rare case of feline EMP that was invasive and recruited T and B cells into the tumor site, showing immunogenicity of PCT, which has not been reported in human and veterinary medicine.

A seven-year-old, castrated male Persian cat was presented to the Veterinary Medical Teaching Hospital, Jeju National University, for the evaluation of a cutaneous mass on the dorsal neck region. The cat had no vaccine history or clinical signs. Physical examination revealed a well-demarcated, hard mass in the interscapular area (Supplementary Fig. 1). There was no peripheral lymphadenopathy.

Complete blood counts and serum biochemistry results were within normal limits, except for an increase in serum amyloid A concentration (SAA, 47.3 μg/mL; reference interval, 0–5 μg/mL). Thoracic radiography revealed a mass with soft tissue opacity located on the caudodorsal region of the scapula. Abdominal ultrasonography and urinalysis were within normal limits. Point-of-care immunoassay for feline immunodeficiency virus antibody, leukemia virus antigen, and heartworm *Dirofilaria immitis* antigen all tested negative.

Fine needle aspiration performed on the subcutaneous mass revealed mild to moderate cellularity of individualized round to oval cells with distinct cytoplasmic cell borders (Fig. 1A). These cells were characterized by a small amount of cytoplasm that was moderately basophilic. The cell size ranged from 2.5 to 4.5 times in erythrocyte diameter. Some cells had a basophilic rim with irregular cytoplasmic borders (Fig. 1B). There were no vacuoles or granules identified within the cytoplasm. Nuclei were round to oval but often were cleaved or highly convoluted with fine to coarsely stippled or clumped chromatin (Fig. 1B, insert). Nucleoli were not distinct in most of the cells, but a few cells had one to two. Occasionally, these cells had an indistinct perinuclear halo. Binucleated cells were sometimes observed. Mitotic figures were found at an average of over 2 per high power field. There were lipid droplets and amorphous basophilic debris in the background. Cytological diagnosis was a round cell tumor with a primary diagnosis of lymphoma. Differential diagnoses included lymphocytic inflammation and other round cell tumors, such as PCT. The mass was surgically resected and subjected to cytopathologic and histopathologic examinations. Impression smears of the mass showed similar cytological features, characterized by medium to large round cells with scant and basophilic cytoplasm (Fig. 1C).

The mass was fixed in 10% neutral-buffered formalin and embedded in paraffin wax. Three micrometer sections were prepared to be stained with hematoxylin and eosin. Microscopically, a variably necrotic dermis contained multifocal to coalescing nodules composed of densely cellular, infiltrative, expansile neoplastic round cells arranged in sheets supported by intact dermal collagen. The coalescing nodules of neoplastic cells were separated by variable large regions of necrosis with frequently scattered necrotic cells and apoptotic bodies. The round neoplastic cells were distinctly bordered, containing small amounts of pale basophilic to amphophilic cytoplasm. Nuclei were round, occasionally indented, centrally located, containing coarsely clumped chromatin and occasional prominent nucleolus. There were eight mitoses per 2.37 mm^2. No evidence of metastasis, including tumor emboli or vascular invasion, was noted. Based on histopathologic findings, a round cell tumor with primary consideration of lymphoma was suggested.

IHC was performed on the serial sections to ascertain the origin of the neoplastic cells, primarily aimed at distinguishing between T and B cell lymphoma. Following hydration and heat-induced antigen retrieval using a citric acid buffer (pH 6.0), endogenous peroxidase activity was blocked by incubating slides in 3% hydrogen peroxide for 20 minutes. Sections were then incubated overnight at 4°C with anti-CD3 and anti-PAX5 antibodies, which were two canonical markers for T and B cells, respectively. Subsequently, sections were incubated with horseradish peroxidase-conjugated secondary antibody for one hour at room temperature. Immunoreactivity was visualized using a 3,3'-diaminobenzidine solution (Dako REAL EnVision kit; Dako, USA) and counterstained with Mayer's hematoxylin. The

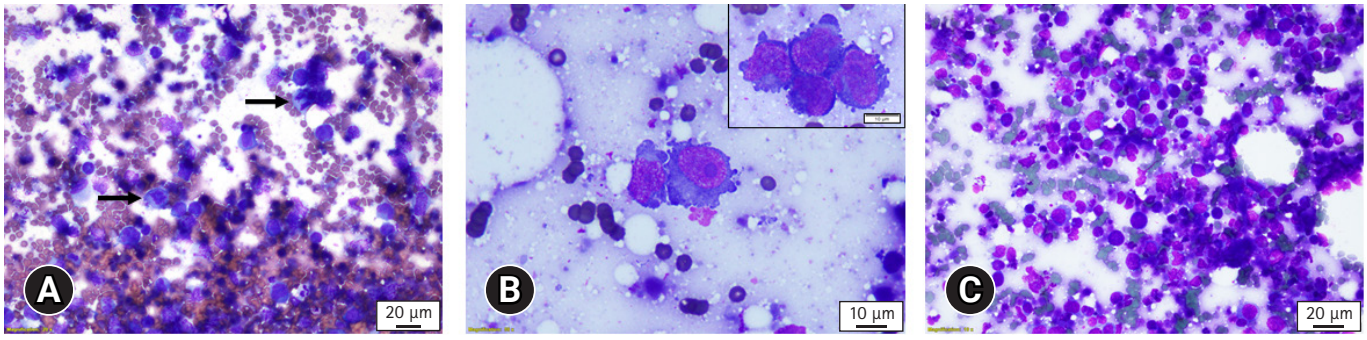

Fig. 1. Cytopathology of extramedullary plasmacytoma in a cat. (A) Fine needle aspirates of the tumor. Individualized medium-sized round cell populations with scant cytoplasm and a high nuclear-to-cytoplasmic ratio are predominant. Note the highly proliferative neoplastic cells evidenced by mitotic figures (arrows). (B) Fine needle aspirates. Note the perinuclear halo area of the neoplastic cells with pseudopodia. Insert indicates a highly cleaved and convoluted nucleus in the neoplastic cells. (C) Impression smears of the tumor resected. Most exfoliated cells are round and medium to large, compared to erythrocyte diameter. (A-C) Diff-Quik stain, scale bars: (A, C) 20 μm, (B) 10 μm, insert 10 μm.

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antibodies and reagents used in this study are present in Supplementary Table 1. As a result, IHC examination revealed that both CD3 and PAX5 stains were negative in the neoplastic cells (Supplementary Fig. 2A and B), suggesting other hematopoietic cell lineages or null cell type lymphoma.

To validate and further immunophenotype this rare type of lymphoma, a paraffin block was retrieved and sent to an independent, external laboratory, and subjected to additional histopathologic and IHC examinations. Similar microscopic descriptions and results were obtained from the secondary institution, primarily considering lymphoma with a differential diagnosis of other round cell tumors (Fig. 2). Meanwhile, round cells in some areas were found to invade subcutaneous adipose tissue (asterisk in Fig. 2A), and margin completeness was not achieved. Consistent with previous IHC results, the neoplastic cells were consistently negative for anti-CD3 and anti-PAX5 (Fig. 3A and B). Mast cell tumor was ruled out based on the negative test results of special staining using toluidine blue (Sigma, USA) (Supplementary Fig. 3A) and IHC stain of anti-c-Kit antibody (Fig. 3C). We next assessed the possibility of plasma cell neoplasm and used an anti-MUM1 antibody, which is a marker for plasma cells [6]. Surprisingly, most of the neoplastic cells had marked nuclear immunoreactivity of MUM1 (Fig. 3D), highly suggesting feline EMP. To corroborate these IHC results, we assessed the lineage differentiation of the B and plasma cells. We tested the immunoreactivity of canonical B cell markers, including CD20 and CD79α, and most importantly CD138, a marker for late stage B cell differentiation into plasma cells. As expected, neoplastic cells were negative for CD20 staining (Fig. 3E). Conversely, neoplastic cells showed moderate to marked positive immunoreactivity against CD79α (Fig. 3F) and CD138 (Fig. 3G), confirming a plasma cell origin. Interestingly, although it has been largely neglected, there were significant numbers of $CD3^+$ T cells within and at the periphery of the neoplasm (Fig. 3H, Supplementary Fig. 3B). Likewise, $PAX5^+$ B cells were infiltrated to a lesser extent into the neoplasm (Supplementary Fig. 3C and D). These tumor-infiltrating lymphocytes were not mitotically active but exhibited scattered and individualized patterns. Based on our extensive cytological, histopathological, and immunohistochemical examinations, the final diagnosis was a feline extramedullary PCT. The patient was treated with cephalexin (20 mg/kg, orally twice daily) for 7 days to prevent potential infections. Despite the surgical removal of the tumor with an incomplete margin, there has been no recurrence or tumor-associated clinical signs detected as of 321 days postoperative.

PCT could be cytologically and histopathologically diagnosed by their distinctive morphologic features, such as eccentrically located pleomorphic nucleus and perinuclear clear zone [4,7]. Histopathologic features also share these cytological features, such as clockface or cartwheel-like nuclear chromatin, although these may not be observed in poorly differentiated neoplastic plasma cells [7,8]. In our case, these typical morphologic features of PCT were rarely identified, but unusual morphologies were noted, including pseudopodia-like cytoplasm, high nuclear-to-cytoplasmic ratio, and mostly centrally located round to

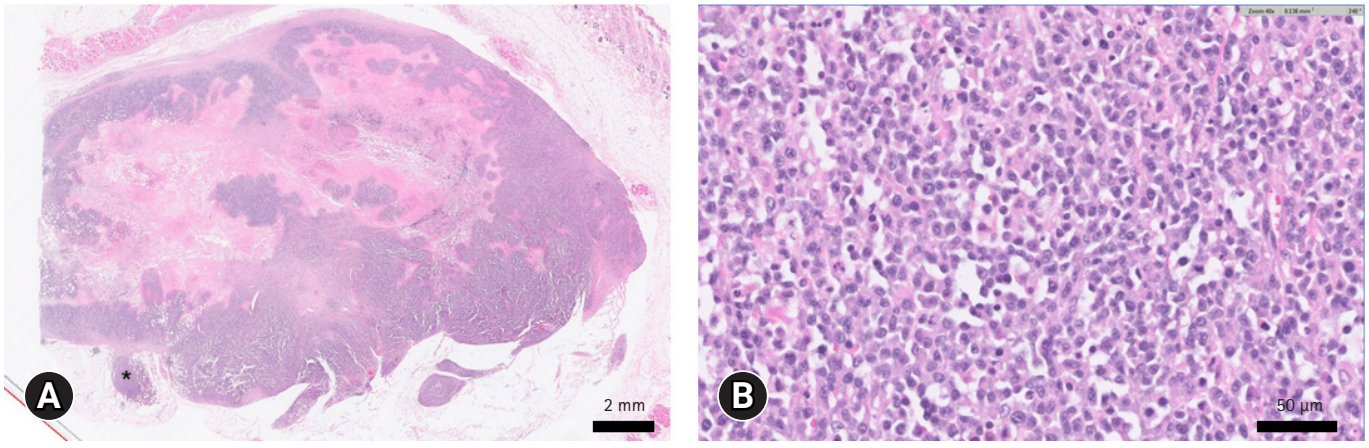

Fig. 2. Histological examination of extramedullary plasmacytoma in a cat. Representative histopathologic images of a skin mass on the dorsal neck of a cat. (A) The solitary mass was confined to the subcutaneous region, with supporting dermal fibrous tissue. Focally extensive coagulative necrosis with hemorrhage and neutrophil inflammation were noted. Neoplastic cells with expansile growth patterns infiltrated surrounding adipose tissue, individualizing the mature adipocytes (asterisk). (B) The mass consisted of densely packed sheets of round neoplastic cells with distinct pale basophilic cytoplasm. Round to oval, occasionally indented nuclei were centrally located. Coarsely stippled chromatin with one to two prominent nucleoli and active mitosis were noted. Hematoxylin and eosin stain, scale bars: (A) 2 mm, (B) 50 μm.

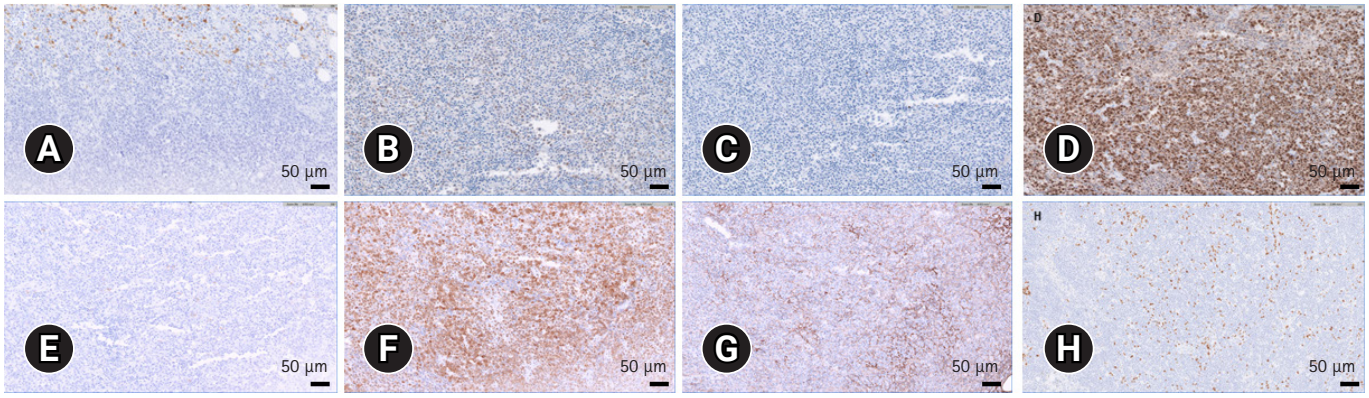

Fig. 3. Immunohistochemical examination for evaluating the origin of neoplastic cells. Extramedullary plasmacytoma in a cat. Representative images of immunohistochemical stains using serial sections. Neoplastic cells are negative for anti-CD3 (A), PAX5 (B), and c-Kit (C) antibodies. Note the strong immunoreactivity of the anti-MUM1 antibiody at the nucleus of the neoplastic cells (D). Neoplastic cells are negative for anti-CD20 (E), but positive for CD79α (F) and CD138 (G) antibodies. Note that patches of neoplastic cells have mild to moderate immunoreactivity of CD138 at their membrane. (H) A significant number of tumor-infiltrating $CD3^+$ T cells are found to be scattered through the neoplasm. Scale bars: (A-H) 50 μm.

pleomorphic nuclei with occasional nucleoli. These features could resemble those of a rare variant of neoplastic lymphocytes, such as lymphoplasmacytic lymphoma [9], which could increase the possibility of misdiagnosis [4]. Although nuclear pleomorphism can be useful as a diagnostic cue for PCT, additional molecular diagnostic workup, such as IHC, is warranted to ascertain the origin of the neoplastic cells and thereby make a definitive diagnosis [6].

In our case, MUM1 was essential for the diagnosis of feline PCT in which combinatorial use of CD79α, CD20, and CD138 also played an important role in assessing B cell origin as well as maturation status. A study reported that histiocytoma could express MUM1 in dogs [6]. Thus, using MUM1 alone might be insufficient to completely differentiate PCT from histiocytoma in cats. Additionally, our case is unlikely to be MUM1 positive lymphoma due to the lack of T and B cell marker expression, although feline lymphoplasmacytic lymphoma of the nervous system could be of the null type but co-express MUM1 and CD44 [10]. Thus, taken together, we strongly recommend practitioners use complete sets of IHC subpanels, including anti-MUM1 antibody, to make a definitive diagnosis of morphologically unusual cases of non-central nervous, extramedullary feline PCT after cytological and histopathological evaluation.

We found a significant number of tumor-infiltrating T cells within feline PCT. Supporting the previous description in which lymphocytes were found in the periphery of the feline PCT [11], our case is believed to add valuable data to the immunological record, suggesting that PCT could be immunogenic in cats. Our results are consistent with the immunogenicity of human [12] and canine PCTs [13], including the spatial distribution of $CD3^+$ T cells. Future studies are warranted to investigate the clinical relevance of T cell infiltration in the feline MRD, which has not been focused on in the field of feline cancer immunology. For example, in humans, the entry of antigen-specific T cells into MM is associated with the acquisition of exhaustive and senescent phenotypes [14].

One case showed that feline EMP may progress to MM [15]. Contrary to a majority of feline PCT cases that are benign and well-demarcated, our case presented an unusual aspect in that neoplastic plasma cells invaded adjacent adipose tissue. The increase in SAA concentration in our case is likely due to intratumoral necrosis and/or tissue injury invaded by the tumor. Currently, it remains completely unclear which criteria could be applied to evaluate the possibility of malignant transformation in feline PCT. The previous case did not describe invasiveness but morphologically noted poorly differentiated neoplastic plasma cells [15]. Our case, at least in part, might have the malignant potential to follow the previous case, given the invasive property and lack of typical plasma cell morphologies. Future studies will be of interest to identify potential factors that drive the malignant transformation of PCT in cats.

We present a rare case of feline PCT that was challenging to make a definitive diagnosis. Our case strongly recommends that practitioners include PCT in their diagnostic routine for round cell tumors in cats, highlighting the importance of a key molecular diagnostic workup to differentiate feline PCT and lymphoma. Finally, our case will be academically valuable in demonstrating the immunogenicity of feline PCT, which may shed light on the future development of novel cancer immunotherapies for feline MRD.

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Conceptualization: Kim MC, Song WJ; Data curation: Kim JH, Kim MC, Song WJ; Funding acquisition: Kim MC; Investigation: Kim JH, Choi SJ, Yun Y; Methodology: Kim MC; Project administration: Kim MC; Supervision: Kim MC; Writing–original draft: Kim JH, Kim MC; Writing–review & editing: all authors.

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Data Availability Statements

All datasets are available in the main manuscript.

Supplementary Materials

Supplementary data are available at https://doi.org/10.14405/kjvr.20240040.

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