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Extranodal γδ-T-cell lymphoma in a dog with leishmaniasis

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Abstract: An 8-year-old intact male mongrel dog with alopecia and weight loss was referred to the Veterinary Faculty of Naples. The dog had pale mucous membranes, enlarged prescapular lymph nodes, and splenomegaly. Laboratory abnormalities included anemia, thrombocytopenia, and hyperglobulinemia. Bone marrow aspirate smears contained numerous *Leishmania* amastigotes and an immunofluorescent antibody titer was strongly positive (1:1280) for leishmaniasis. The dog was treated with a combination of meglumine antimoniate and allopurinol for 60 days and showed clinical improvement. Two months after the end of treatment the dog was again referred because of relapse of leishmaniasis and the presence of a firm subcutaneous mass on the medial right thigh. Based on cytologic examination of fine needle aspirates of the mass, a diagnosis of large-cell lymphoma was made. Flow cytometry of tumor cells revealed γδ-T-cell lymphoma with a CD5+, CD3+, TCRγδ+, CD4−, CD8−, CD45RA+ immunophenotype. Using nested PCR, amastigotes were not detected in the neoplastic tissue. An association between leishmaniasis and hematopoietic tumors has been described rarely. γδ-T cells may be involved in the host response to this parasite, and prolonged antigenic stimulation and chronic immunosuppression (typical of leishmaniasis) play a crucial role in the etiopathogenesis of T-cell lymphoma.

Case Presentation

An 8-year-old intact male mongrel dog with alopecia and weight loss was referred to the Veterinary Faculty of Naples. Physical examination revealed pale mucous membranes, enlargement of prescapular lymph nodes, and splenomegaly. Three milliters of blood were collected by jugular venipuncture into EDTA-containing vials and submitted for automatic analysis (SEAC-Genius/S/VET-Hemat 8, Calenzano, Florence, Italy) of CBC and platelet count. Evaluation of blood cell morphology and differential leukocyte counts were performed on smears stained by routine methods. A serum biochemical profile was performed on blood samples (7 mL) collected into tubes without anticoagulant and centrifuged at 200 *g* to obtain serum (Analyzer Medical System, Rome, Italy, SABA 18). Hematology and clinical chemistry abnormalities included microcytic-normochromic anemia, thrombocytopenia, hyperproteinemia, hypoalbuminemia, and hyperglobulinemia (Table 1). Bone marrow aspirate smears revealed numerous *Leishmania* sp. amastigotes. An immunofluorescent antibody titer (IFAT) was strongly positive (1:1280; cutoff 1:80) for *Leishmania infantum*. The dog was treated with a combination of meglumine antimoniate (50 mg/kg SC, BID) and allopurinol (10 mg/kg PO, BID) for 60 days. At the end of the treatment period, the dog was clinically recovered and had CBC and biochemistry values within the reference intervals for our laboratory.

Two months after the end of therapy, the dog was referred again due to a relapse. Physical examination findings included exfoliative dermatitis, lymphadenomegaly, and a painless, irregular walnut-sized mass in the subcutaneous tissue on the medial right thigh (Figure 1). Results of a CBC included severe, normocytic-normochromic, regenerative anemia; eosinophilia; and hyperproteinemia (Table 1, day 120). Bone marrow aspirate

Table 1. Hematologic and biochemical data from a dog with leishmaniasis (day 1) and subsequently extranodal lymphoma (day 120).

Analyte	Day 1	Day 120	Reference Interval*
RBC ($\times 10^6/\mu L$)	4.13	4.41	5.50–7.90
HGB (g/dL)	8.0	10.7	12.0–18.0
HCT (%)	23.2	29.8	37.0–55.0
MCV (fL)	56	68	60–76
MCH (pg)	19.5	24.3	20.0–27.0
MCHC (g/dL)	34.7	36.0	32.0–38.0
Reticulocytes (/μL)	Not done	273,420	< 60,000
WBC ($\times 10^3/\mu L$)	5.0	6.3	6.0–16.0
Band neutrophils ($\times 10^3/\mu L$)	0	0	0–0.3
Segmented neutrophils ($\times 10^3/\mu L$)	3.75	3.46	3.0–11.5
Lymphocytes ($\times 10^3/\mu L$)	0.90	1.52	1–4.8
Monocytes ($\times 10^3/\mu L$)	0.10	1.89	0.1–1.3
Eosinophils ($\times 10^3/\mu L$)	0.25	1.13	0.1–1.3
Basophils ($\times 10^3/\mu L$)	0	0	0
Platelets ($\times 10^3/\mu L$)	124	312	200–400
Urea (mg/dL)	35.0	34.5	20–35
Creatinine (mg/dL)	0.6	0.8	< 1.3
Total protein (g/dL)	8.4	9.2	6.0–7.4
Albumin (g/dL)	1.55	2.45	2.6–3.3
Globulins (g/dL)	6.85	6.75	3.4–4.1
Alanine aminotransferase (U/L)	24.2	25.0	3–25

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evaluation demonstrated a slightly increased myeloid to erythroid ratio of 2.25 (reference interval 0.6–2.0) with eosinophilic hyperplasia and the presence of numerous amastigotes. Fine needle aspiration cytology of the mass showed large round cells with cleaved nuclei, finely granular chromatin, large nucleoli, and basophilic cytoplasm containing small and prominent vacuoles (Figure 2). Based on the cytologic features, a diagnosis of large-cell lymphoma was made. Biopsy was performed but the owners declined surgical excision of the mass.

Figure 1. Irregular walnut-sized mass (arrow) on the medial thigh of a dog.

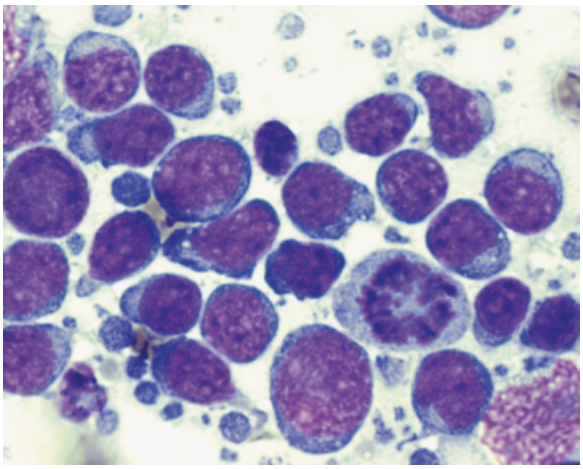

Figure 2. Fine needle aspirate of the subcutaneous mass. A population of lymphoid cells with large and sometimes cleaved nuclei, finely granular chromatin, and basophilic cytoplasm is seen. An atypical mitotic figure is also evident. The diagnosis was large-cell lymphoma. May-Grunwald-Giemsa, × 100 objective.

Blood, bone marrow, and tumor cell immunophenotype was obtained by flow cytometric analysis using specific canine monoclonal antibodies against CD3 (CA17.2A12); CD5 (DH3D); TCRαβ (CA15.8G7); TCRγδ (CA20.8H1); CD4 (CA13.1E4); CD8 (CA9JD3) for T lymphocytes; CD21 (CA21D6) for B lymphocytes; CD45RA (CA41D3) for B lymphocytes and T naïve lymphocytes; and CD45 (CA12.10C12), a panleukocyte marker. All antibodies were obtained from Peter F. Moore (University of California, Davis) with the exception of CD5, which was provided as a gift by VMRD (Pullman, WA, USA). Blood and bone marrow cell immunophenotype was normal (no neoplastic population identified). Neoplastic lymphoid cells in the tumor had a CD5+, CD3+, TCRγδ+, CD4−, CD8−, CD45RA+ phenotype.[1]

Cytologic examination of a popliteal lymph node aspirate showed no abnormalities while an aspirate of the superficial cervical lymph node showed reactive hyperplasia; these lymph nodes were not clinically enlarged. On histologic examination of the biopsy specimen, the subcutaneous tissue appeared infiltrated by a diffuse proliferation of atypical lymphoid cells having enlarged, basophilic, sometimes cleaved nuclei; visible nucleoli; and scant, pale, finely granular cytoplasm (Figure 3). Neoplastic proliferation did not show well defined margins and extended to dermis without reaching the upper epidermis.

Neoplastic lymphoid tissue was cultured using Evans' Modified Tobie's Medium (prepared in-house)[2]

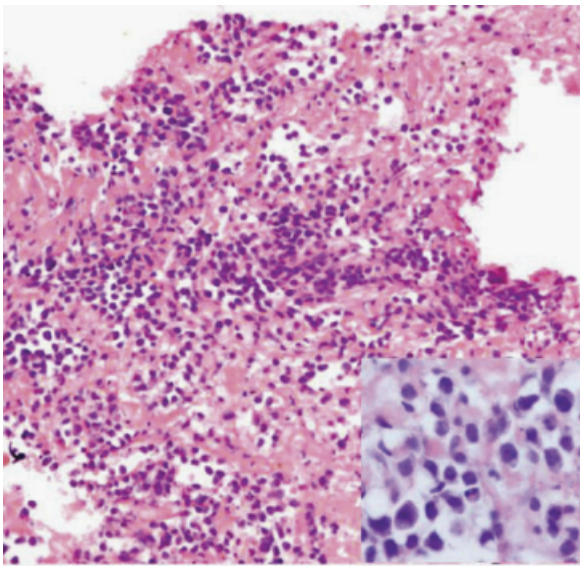

Figure 3. Histologic section of the subcutaneous mass. Atypical lymphoid cells are infiltrating the subcutaneous tissue. H&E, × 20 objective. Inset: Neoplastic cells with pleomorphic nuclei are evident. H&E, × 40 objective.

and processed by nested-PCR[3] to evaluate the potential presence of amastigotes in the neoplastic mass; the results of both analyses were negative. The dog died 20 days later because of trauma sustained after being hit by a car.

Discussion

Canine leishmaniasis is characterized by chronic progression of viscerocutaneous signs, which occur in fewer than 50% of infected dogs.[4–6] The most common signs are lymphadenopathy, skin lesions, weight loss, mucosal pallor, ocular lesions, and polyuria-polydipsia associated with renal involvement. Laboratory findings comprise increased serum immunoglobulin concentration, decreased albumin/globulin ratio, moderate normocytic normochromic anemia, moderate neutrophilia, and azotemia.[1] Canine leishmaniasis is frequently associated with other infections (ehrlichiosis, babesiosis) and rarely with neoplastic diseases, including hematopoietic tumors.[1] In our experience, the prevalence of the multicentric form of lymphoma in dogs suffering from leishmaniasis is 1:300 cases (unpublished data). Canine extranodal lymphoma is rare and the dermal/subcutaneous form has a survival of only 4 months, even after chemotherapy.[7]

γδ-T-cells represent < 1–2% of peripheral blood canine T lymphocytes. γδ-Neoplasms have been described previously in the dog, in granular lymphocyte chronic lymphoid leukemia, hepatosplenic lymphoma, nonepitheliotropic cutaneous lymphoma, and epitheliotropic cutaneous lymphoma.[8–11] Pagetoid reticulosis is considered to be an exclusively γδ-T lymphoma.[12,13] In humans, γδ-T-cell lymphomas are a unique clinicopathologic entity, of which hepatosplenic γδ-T-cell lymphoma is the prototype. These neoplasms have also been described in other extranodal sites showing high morphologic heterogeneity.[14] It is interesting to note the existence of a human γδ-T-cell subcutaneous lymphoma characterized by a population of pleomorphic cells and with a CD3+ and CD4− CD8± phenotype, which is very similar to the form of lymphoma described in this case.[15]

γδ-T lymphocytes can be involved in the first host response during bacterial and parasitic infections.[16] They have been studied in human and murine leishmaniasis, but their function has not been well defined. Curiously, γδ-T cells accumulate in the blood and in cutaneous lesions of humans infected with *Leishmania* parasites.[17,18] This indicates that γδ-T cells could be involved in the host response to this parasite. However, in the present case it was not possible to demonstrate the presence of the parasite in neoplastic tissue. It is well known that in murine, human and, probably, canine leishmaniasis, the CD4+ subtype Th1 response induces resistance against development of the disease. In contrast to this, the CD4+ subtype Th2 induces susceptibility.[19,20]

Previous studies in Balb/c mice infected with *Leishmania major* in which a Th1 or Th2 response was induced, demonstrated that an increase in γδ-T cells was modulated by the presence of CD4+ T cells, subtype Th2.[21] The dog in this case developed a typical Th2 response characterized by severity of the clinical signs, bone marrow eosinophilic hyperplasia, a high IFAT titer, and relapse. Eosinophilic hyperplasia could represent a response to the bone marrow colonization of *Leishmania infantum*, but is an usual finding in dogs suffering from leishmaniasis.[22] Prolonged antigenic stimulation and chronic immunosuppression (a phenomenon typical of leishmania infection) plays a crucial role in the etiopathogenesis of T-cell lymphoma.[16] In conclusion, these mechanisms could result first in an increase in γδ-T cells and then in their malignant transformation. This hypothesis needs to be investigated further.

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