We identified a dog with large granular lymphocytic leukemia and cutaneous lymphoma that exhibited constitutive expression of interleukin-2 (IL-2) receptors by the leukemic peripheral blood lymphocytes. The leukemic cells phenotypically resembled natural killer (NK) cells, and their surface IL-2 receptors were functional, as determined by the capacity to bind human recombinant IL-2 with high-affinity resulting in the transduction of proliferation signals and in the development of lymphokine-activated killer cell activity. These cells produced IL-2 spontaneously, and they may have maintained their proliferative state through an IL-2-dependent autocrine growth pathway. Our results indicate that neoplastic lymphocytes of syndromes that involve circulating leukemic cells with dermoptropism can originate from NK-like cells. Additionally, the data also suggest that proliferative conditions such as these may be the result of the aberrant production of IL-2. Further, this case illustrates the potential for the use of hematopoietic malignancies in the dog as a suitable animal model for immune targeting of IL-2 receptors as a novel treatment approach for similar malignancies of human beings.

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MATERIALS AND METHODS

Case report. A 10-year old neutered female mixed-breed dog weighing 19.5 kg, was referred to the Veterinary Medical Teaching Hospital at the School of Veterinary Medicine, University of Wisconsin for evaluation of a generalized pruritic skin disorder that had progressed during the 5 months before presentation. The dog had been treated with prednisone (0.5 mg/kg daily) for 2 weeks before evaluation. The physical examination showed an alert, afebrile dog with generalized, diffuse cutaneous erythema, patchy alopecia, scale, crusted, and focal ulceration. Moderate enlargement of several peripheral lymph nodes was also detected. The complete blood count showed leukocytosis (48,000 WBC/µL) due to lymphocytosis (41,310 lymphocytes/µL; normal: 1,000 to 4,800/µL) with a normal distribution of granulocytes (5,832 neutrophils/µL; 972 eosinophils/µL) and monocytes (486/µL). The lymphocytes were moderately large, with abundant basophilic cytoplasm and azurophilic granules (Fig. 1). Nuclei contained clumped chromatin with an occasional prominent nucleolus. The hemoglobin concentration (15.9 g/dL), packed cell volume (44%), red blood cells (RBC) count (6,76 × 10^6/µL), and RBC indices (mean corpuscular volume, 69 fl; mean corpuscular hemoglobin, 24 pg; mean corpuscular hemoglobin concentration, 36%) were all normal for the canine. Thrombocytosis expression of IL-2R and the development of disease is better understood. In this type of leukemia, abnormal expression of the IL-2R α subunit (p55, Tac) is constitutively induced by transactivation of the promoter of the IL-2R α gene by the 40 kDa tax protein, encoded within the HTLV-l genome.8 The abnormal expression of these receptors on neoplastic hemolymphatic cells is associated with malignant growth, as immune targeting of the IL-2R with a toxin such as diphtheria A chain can arrest growth of these cells.9,10

We investigated IL-2R expression on leukemic cells from a dog referred to our clinic for therapy for cutaneous lymphoma. These studies were done to determine if this animal’s spontaneous cancer resembled that of some human hematopoietic malignancies in its expression of IL-2R and to investigate whether the proliferation of these cells could be driven by IL-2. Our data show that the leukemic cells from this dog were natural killer (NK)-like in origin. These cells proliferated and showed cytotoxic activity in response to IL-2, similar to NK cells in other species. This suggests that cutaneous lymphoma and associated leukemia in the dog can be of a non-T-cell phenotype, and also clarifies a potential future role for canine leukemia as a model for IL-2R targeted immunotherapy in human hematopoietic malignancies.11-13

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nucleated cells in focal areas. The erythroid and myeloid series were present in all stages and demonstrated progressive maturation. Overall, the myeloid:erythroid (M:E) ratio was considered within normal limits and adequate numbers of megakaryocytes were present. A fine needle aspirate of an enlarged peripheral lymph node also contained atypical lymphocytes similar to those in the blood and bone marrow. Abdominal radiographs showed hepatomegaly, while thoracic radiographs and protein electrophoresis of the dog’s serum were unremarkable. Several skin punch biopsies submitted for histologic evaluation were diagnosed as cutaneous malignant lymphoma with minimal epitheliotropism (Fig 2).

Heparinized venous blood was drawn for in vitro lymphocyte studies and the dog was treated with a conjugate of polyethylene glycol (PEG)-asparaginase (30 IU/kg intramuscularly; Enzon, Inc, Piscataway, NJ) once per week for 6 weeks. This therapy resulted in a partial decrease in the number of abnormal circulating lymphocytes (12,095/μL) without improvement in skin lesions. Therapy was then changed to intravenous doxorubicin (30 mg/m²; Adria Laboratories, Columbus, OH) every 3 weeks for a total of

(532,000 platelets/μL) was present, and the plasma protein concentration (7.1 g/dL) was normal. The serum biochemical profile indicated elevated alkaline phosphatase activity (649 IU/L).

Cytologic evaluation of a bone marrow aspirate showed diffuse infiltration by atypical lymphoid cells morphologically similar to those in the peripheral blood and comprising 20% to 70% of the population of large neoplastic lymphocytes. Infiltration of the deep epidermis is seen, although this feature was rare. Original magnification = 6,900.

![Peripheral blood leukemic cells from the affected dog. (A) Wright's stained peripheral blood lymphocyte showing cytoplasmic granules and clumped nuclear chromatin. Original magnification = 500. (B) Ultrastructure of malignant peripheral blood lymphocyte. Original magnification = 11,500.](image)

![Cutaneous lymphoma from the affected dog. (A) Hematoxylin and eosin stained section of a skin biopsy. Immediately deep to the epidermis, there is diffuse intradermal infiltration by a homogeneous population of large neoplastic lymphocytes. Infiltration of the deep epidermis is seen, although this feature was rare. Original magnification = 300. (B) Ultrastructure of malignant lymphocytes in the skin, prepared from formalin-fixed tissue. Original magnification = 6,900.](image)
four doses. Vincristine sulfate (0.7 mg/m² intravenously; Lyphomed, Deerfield, IL) was given with the doxorubicin in the last two cycles. During this time, the atypical cellular lymphocytosis progressed, and the bone marrow continued to be infiltrated with malignant lymphocytes. Similarly, the skin lesions were unresponsive to the combination of doxorubicin/vincristine. The dog's owners declined further therapy but consented to additional phlebotomy to facilitate continuation of in vitro studies of the dog’s peripheral blood lymphocytes.

Cells. Peripheral blood lymphocytes (PBL) were obtained from heparinized venous blood of the canine patient and four normal adult canine donors by separation over a ficoll-hypaque (Histopaque-1077, Sigma Chemical Co, St Louis, MO) discontinuous density gradient (specific gravity 1.077), as described.5,17 Some cells from the canine patient were cryopreserved immediately on isolation from the peripheral blood. All phlebotomy procedures were performed in accordance with a protocol approved by the University of Wisconsin Institutional Animal Care and Use Committee. The isolated cells were washed three times in Hank’s Balanced Salt Solution and were resuspended in RPMI-1640 supplemented with 10% vol/vol heat-inactivated fetal calf serum, 2 mmol/L sodium pyruvate, 2 mmol/L L-glutamine, 10 mmol/L HEPES buffer, penicillin (100 U/ml), streptomycin (100 µg/ml), and L-glutamine (100 µg/ml). The viability of the isolated PBL, as assessed by trypan blue dye exclusion, was routinely greater than 98% at the time of culture.

A canine thyroid adenocarcinoma cell line (CTAC),18,19 (provided by Dr John Jardine, Houston, TX) was used as a cell target. This line was grown in supplemented RPMI-1640 as an adherent line.

Kit-225 cells, an IL-2-dependent transformed human T cell line,20 were a gift of Dr T. Uchiyama (Kyoto, Japan). The cells were maintained in continuous culture in supplemented RPMI-1640 containing 2 mmol/L human recombinant IL-2.

Antibodies. Murine monoclonal antibodies (MoAbs) 12.125 (anticanine CD4),1,22 4.78 (anticanine CD8),21,22 and 1A4, an anticanine thymocyte antibody23 as ascites, were used to label fresh and cultured PBL from the patient and control dogs. A polyclonal rabbit antibody (Dako, Carpinteria, CA) that recognizes canine CD36 was a gift of Dr T. Uchiyama (Kyoto, Japan). The cells were maintained in continuous culture in supplemented RPMI-1640 containing 2 mmol/L human recombinant IL-2.

Flow cytometric analyses. Flow cytometric analyses of PBL from the affected and control dogs were performed on a FACScan flow cytometer (Becton Dickinson, San Jose, CA) using a human monoclonal antibody (MoAb CA15.8G7) specific for the T cell receptor (MoAb CA15.8G7),* anticanine FACScan flow cytometer (Becton Dickinson, San Jose, CA) using of PBL from the affected and control dogs were performed on a

Lymphocyte proliferation assay. Mitogens were added to individual cultures at the indicated concentrations, and included: phytohemagglutinin (PHA-P, 5 µg/ml; Sigma Chemical Co) and human recombinant IL-2 (100 U/ml). Controls consisted of PBL cultured in medium. The proliferative response of canine PBL to mitogens was determined by incorporation of 3H-thymidine to DNA, as described.17

IL-2 receptor expression. IL-2 receptors were detected by flow cytometry using biotinylated human recombinant IL-2 and avidin conjugated to fluorescein isothiocyanate (FITC) (Fluorokine IL-2 Receptor Kit, R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions with the following modifications. Lymphocytes were washed and suspended in buffer at 4 × 10^6 cells/ml, and 50 µl (containing 2 × 10^6 cells) placed in staining tubes. Twenty microliters of stock biotinylated human recombinant IL-2 (6.6 × 10^4 mol/l) were added to each tube and allowed to incubate for 60 minutes at 4°C. Cells were then washed twice in 1 ml buffer and resuspended in 200 µl buffer. Twenty microliters of the avidin-FITC reagent were added to each tube and allowed to incubate for 30 minutes at 4°C in the dark. Cells were then washed twice more in buffer to remove unbound avidin-FITC and resuspended in 1 ml of phosphate buffered saline (PBS) for flow cytometric analysis with a FACScan flow cytometer (Becton Dickinson). The negative control consisted of cells reacted with avidin-FITC without prior incubation with biotinylated IL-2. The positive control consisted of PBL from a normal dog activated with PHA (5 µg/ml) for 3 days, a time when PHA-activated normal canine PBL maximally express IL-2 receptors.21 In some assays, 100-fold molar excess of unlabeled human recombinant IL-2 (10^-4 mol/l; 20 µl) was added 60 minutes before incubation with biotinylated IL-2 to demonstrate the specificity of the biotinylated IL-2.

Immunohistopathology. Cryopreserved cells were used and were thawed at 37°C, centrifuged at 400 × g for 10 minutes and washed twice in sterile PBS. The cell pellets were lysed in a buffer containing 300 mmol/L NaCl, 50 mmol/L Tris (pH 7.6), 0.5% Triton X-100, 1 mmol/L N-ethyl-maleimide, 0.03 mmol/L aprotinin, and 0.5 mmol/L leupeptin. Culture supernatants of the dog’s leukemic cells were up to 10-fold longer than canine controls, as described.5,17,18 For these experiments, CTAC cells were cultured in medium. The proliferative response of canine PBL to mitogens was determined by incorporation of 3H-thymidine to DNA, as described.17

Immunohistochernical staining. Formalin-fixed skin biopsies were paraffin-embedded and 5 µm sections stained by a streptavidin-
horseradish peroxidase immunohistochemical method (Zymed, South San Francisco, CA), as described. Antigen retrieval employing a trypsin digestion technique for CD3ε and microwave treatment for Igα were used to enhance detection of these antigens before immunostaining.

RESULTS

Proliferation assays. The fresh PBL obtained from the leukemic dog behaved like competent cells (ie, activated lymphocytes that were arrested in G1) in their response to IL-2 (100 U/mL; 4.2 x 10⁻¹⁰ mol/L), in that the response peaked earlier and was of a greater magnitude than the response to PHA (Fig 3A). The control PBL from a healthy dog demonstrated the typical responses to IL-2 and PHA in which the proliferation stimulated by PHA peaked earlier and was of a greater magnitude than that induced by IL-2 (Fig 3B).

The capacity of the dog’s leukemic PBL to proliferate in response to IL-2 was likely mediated through IL-2 receptors. We performed standard dose response curves to IL-2 to determine whether the response of these cells to the cytokine followed the expected biologic behavior mediated through IL-2 receptors. The dog’s leukemic PBL achieved maximal proliferation in response to IL-2 at a concentration of 10⁻¹⁰ mol/L, suggesting saturation of IL-2 receptors at this dose (Fig 4A). This response was similar to that of canine or human lymphoid cells that express high affinity IL-2 receptors.

To further establish the requirement for IL-2 by the leukemic cells from this dog, aliquots of fresh PBL were propagated for 2 months in the presence of IL-2 before they eventually died. Cells were passaged at 5 x 10⁶ cells/mL every 3 days, and fresh IL-2 (100 U/mL) was added at every passage. After 1 month in culture, viability of cells, assessed by trypan blue exclusion, was ≈80%. At this time, dependency of these cells on IL-2 for growth was evaluated. Cultured cells seeded into 96-well plates continued to divide only if IL-2 (100 U/mL) was provided (Fig 4B). Additional efforts to immortalize the leukemic PBL by stimulation with irradiated PBL feeder cells from another dog or PHA were unsuccessful.

IL-2 receptor expression. Specific binding of IL-2 by IL-2 receptors on fresh leukemic PBL was demonstrated by flow cytometry using biotinylated human recombinant IL-2 and avidin-FITC (Fig 5A). IL-2 receptors appeared to be expressed by all cells in this bulk population in a homogeneous fashion as suggested by the positive fluorescence of the entire cell sample with a narrow distribution of fluorescence intensity (Fig 5A). Pretreatment with a 100-fold molar excess of unlabeled IL-2 significantly reduced the fluorescence intensity of the cells incubated with biotinylated IL-2 and avidin-FITC, indicating the binding of IL-2 to these cells was specific (Fig 5A).

As was expected, resting normal canine PBL did not bind IL-2 (Fig 5B), indicating they lacked surface IL-2 receptors. But following activation of these PBL with PHA for 3 days, IL-2 receptors were easily detectable (Fig 5C). As with the leukemic PBL, this staining could be blocked by preincubating the cells with a 100-fold excess of unlabeled IL-2 (Fig 5C). Consistent with polyclonal T cell activation induced by PHA, the fluorescence intensity of the positively staining PHA-activated normal PBL varied widely as compared with the relatively uniform fluorescence intensity of the positively stained (IL-2-bound) leukemic PBL. This suggested that the IL-2R positive PBL population in the leukemic dog was clonal or oligoclonal.

IL-2 production. Our data show that in short-term cultures of leukemic PBL propagated for 1 month in vitro, the IL-2-stimulated leukemic cells continued to proliferate for 8 days (Fig 4B), despite the fact that the exogenous IL-2 was likely depleted at this time. We assessed the capacity of the leukemic PBL to produce IL-2 in vitro as an indicator
of the cells’ potential ability to produce IL-2 in vivo. Therefore, we examined whether cell-associated IL-2 was present in fresh (cryopreserved) leukemic cells, in cultured leukemic cells, or in supernatants from the fresh leukemic PBL by immunoblotting. A polyclonal rabbit antihuman IL-2 antibody recognized a band of approximately 16.5 kDa in supernatants from fresh unstimulated leukemic PBL from the affected dog cultured for 24, 48, or 72 hours (Fig 6, lanes 1-3). This IL-2 band in the supernatants was barely detectable after 24 hours, but increased in intensity after the cells were cultured for 48 or 72 hours. These data suggest that the cells were secreting IL-2 into the culture medium and that the cytokine accumulated over the culture period. A band of approximately 15 kDa that comigrated with human recombinant IL-2 (lane 7) was present in whole cell lysates from the fresh leukemic PBL (lane 4) and from the cultured leukemic cells that were grown in the presence of IL-2 (lane 5), but not in human Kit-225 cells grown in IL-2 (lane 6), suggesting the IL-2 was elaborated by the cells, rather than only internalized. The discrepancy in the apparent molecular weight of the IL-2 band obtained from the supernatants compared with that of the cell lysates and recombinant IL-2 could be due to the need for loading of increased amounts of protein in the supernatants compared to cell lysates and recombinant IL-2 resulting in slightly slower migration through the gel. Alternatively, these results could be due to differences in glycosylation and phosphorylation between secreted and cell-associated IL-2.

**Immunophenotype.** To determine the developmental origin of the malignant population in this leukemic dog’s PBL, the cells were analyzed by indirect immunofluorescence with canine T and B cell markers. These fresh leukemic cells stained positive with MoAb 1A1 reported to recognize T cells and NK-like cells in the dog, however, they did not express CD4 or CD8. There was no change in the immunophenotype of these PBL after culture with IL-2 for 4 weeks. Furthermore, >98% of these CD4⁺, CD8⁻ cells obtained directly from the dog or after culture were positive for IL-2R (Fig 7). In contrast, the majority of fresh PBL from two normal canine donors were a mixed lymphocyte population composed of CD4⁺ and CD8⁻ cells, present approximately in a 2:1 ratio. Culture of these normal PBL in the presence of IL-2 (100 U/mL) for 3 days did not alter the immunophenotype distribution appreciably. In the same experiments, all PBL stained positive for 1A1 (Fig 7). Fresh leukemic PBL that had been cryopreserved were analyzed further. These cells still did not express CD4 or CD8, and in addition, they were negative for canine CD3, T cell receptor αβ and γδ subunits, and Igα, suggesting a null cell or NK cell origin. Specific NK markers have not been characterized in the dog.

The malignant lymphocytes found in the skin biopsies also were CD3 and Igα negative. Other canine leukemia antigens are destroyed in the process of formalin fixation and paraffin-embedding, making them unavailable for study in the preserved sections used for immunohistochemistry.

**Cell-mediated lysis.** To establish whether the IL-2R on the leukemic cells from this dog were functional, and to examine the possibility these cells may have originated from an NK-like cell, we examined their lymphokine-activated killer (LAK) activity in response to IL-2. Lysis of CTAC target cells mediated by leukemic PBL cultured in IL-2 (100 U/mL), fresh PBL from three normal dogs, and corresponding normal dog PBL cultured in IL-2 (100 U/mL) for 3 days (ie, 3-day LAK cells°), was examined (Fig 8). Fresh PBL from the normal dogs mediated only low level cytotoxicity against the CTAC target. In vitro generated LAK cells from these same dogs mediated three- to 14-fold greater cytotoxicity (Fig 8). Leukemic PBL, which had been in culture with IL-2 (100 U/mL) for 4 weeks, mediated cytotoxicity of comparable magnitude to the normal dogs’ 3-day LAK cells (Fig 8). Fresh leukemic cells were unavailable for cytotoxicity experiments.

**DISCUSSION**

We have described a canine cancer patient with cutaneous lymphoma and a lymphoid leukemic cell population that
IL-2 receptors on canine NK leukemic cells

Fig 5. IL-2 receptor expression by PBL from the leukemic dog and PBL from a control dog. (A) Unstimulated, fresh PBL from the leukemic dog were incubated in the absence or presence of biotinylated hrIL-2 (6.6 x 10^{-8} mol/L) (IL2-B) and avidin-FITC. A 100-fold molar excess of unlabeled hrIL-2 was used to compete the binding of the biotinylated IL-2 (IL2 + IL2-B) to demonstrate the specificity of this response. Similar results were obtained in one additional experiment. Mean fluorescence intensity (MFI): no IL2 = 6.84; IL2-B = 67.83; IL2 + IL2-B = 32.36. (B) Fresh unstimulated PBL from a normal dog were stained as in (A). MFI: no IL2 = 4.42; IL2-B = 7.93; IL2 + IL2-B = 4.86. (C) PBL from the normal dog shown in (B) were stimulated with PHA (5 pg/mL) for 3 days and stained as in (A). MFI: no IL2 = 12.95; IL2-B = 166.23; IL2 + IL2-B = 63.72. Relative fluorescence is shown on the X axis (logarithmic) and relative cell number on the Y axis (linear).

Fig 6. IL-2 production by PBL from the leukemic dog. The presence of IL-2 in culture supernatants or in whole cell lysates was examined by immunoblotting. Culture supernatants from freshly isolated, unstimulated leukemic PBL from the affected dog were collected after 24 hours (lane 1), 48 hours (lane 2) or 72 hours (lane 3). The accumulation of cytosolic IL-2 protein was examined in freshly isolated (cryopreserved) leukemic cells from the affected dog (lane 4), in leukemic cells from the affected dog that had been cultured in the presence of IL-2 for 4 weeks (lane 5) or in the human IL-2-dependent cell line Kit-225 cultured in the presence of IL-2 for 3 weeks (lane 6). Lane 7 contained 5 ng of human recombinant IL-2 used as a positive control. The presence of IL-2 was detected by immunoblotting using chemiluminescence (lanes 1 through 3) or alkaline phosphatase (lanes 4 through 7) systems. Similar results were obtained in two additional experiments.
Fig 7. IL-2 receptor expression by canine CD4−CD8− leukemic NK-like cells. Indirect flow cytometry was used to detect IL-2 receptors (IL-2R) on fresh (cryopreserved) leukemic canine PBL by binding of biotinylated human recombinant IL-2 and fluorescence of bound avidin conjugated to fluorescein isothiocyanate (FITC) (X axis A, B, and C). Staining of cells for lymphocyte markers CD4 (A), CD8 (B), and 1A1 (against canine thymocytes and NK cells) (C) is shown on the Y axis using murine MoAbs detected by fluorescence of bound goat antimouse Ig conjugated to phycoerythrin (PE). Data are shown as 20% probability contour plots.

Fig 8. Cell lysis mediated by leukemic and normal canine PBL. Cell lysis mediated by leukemic canine PBL cultured with IL-2 (100 U/mL) for 1 month (III), fresh PBL from three normal dogs (○), and corresponding normal dog PBL cultured with IL-2 (100 U/mL) for 3 days (ie, 3-day lymphokine-activated killer cells [LAK cells]) (□) was measured in a 4-hour 51Cr release assay using a canine thyroid adenocarcinoma cell line target. The concentration of effector cells was adjusted to give a final effector/target cell (E:T) ratio of 100:1, 50:1, and 25:1. 51Cr release data from quadruplicate samples were converted to lytic units where one lytic unit is defined as the number of effector cells required to cause 20% lysis of 5 × 10⁶ target cells per 10⁷ effector cells used.

anti-Tac was not possible due to lack of cross-reactivity of these antibodies with canine cells. It was important to determine whether expression of these receptors was directly related to the pathogenesis of the lymphoid cancer or a by-product of the malignant transformation of the cells. To begin to address this important point, we analyzed the capacity of IL-2 to induce physiologic responses in these cells.

The leukemic cells proliferated in response to IL-2 and, in short-term culture, exhibited a dependency on it for growth. However, in the same short-term cultures, the leukemic cells stimulated with IL-2 continued to divide at a time when the exogenous IL-2 was likely to be depleted from the cultures. Our results show that, based on immunoreactivity with anti–IL-2 antibody, these cells elaborated small amounts of IL-2 immediately on isolation from the dog and continued to do so after they had been in tissue culture for a period of 1 month in the presence of IL-2. It is possible that the IL-2 gene could have been activated inappropriately in the leukemic cells providing a selective growth advantage for leukemic PBL expressing IL-2R. However, these cells clearly exhibited an initial requirement for exogenous IL-2 to proliferate in vitro. The small amounts of IL-2 that were detectable in the culture supernatants and in the cell lysates suggest that this dependence on exogenous IL-2 could have been due to insufficient amounts of autocrine IL-2 production by the leukemic cells in vitro. In addition to promoting cell cycle progression, the exogenous IL-2 provided for the cells at the onset of the cultures might have induced further expression of IL-2 receptors and lowered the IL-2 response threshold of the cells. Alternatively, the cells may have been secreting a protein that was immunologically similar to, but distinct from IL-2 that acted as a cofactor for NK-like cell growth only in the presence of exogenous IL-2. Nevertheless, the data suggest that a possible IL-2 autocrine loop may
have been responsible, at least in part, for the growth of these cells in vivo. Failure of the leukemic PBL to continue to proliferate in vitro over the long-term, despite continued supplementation of the culture medium with IL-2, could indicate a need for other essential stimulatory or supportive molecules not present in the cultures. The propagation of human cutaneous T cell lymphomas and human Sézary cells, circulating malignant T lymphocytes associated with cutaneous lymphoma, in vitro have also been difficult.25–27 Recently, a 28-kD protein, Sézary T cell activating factor (SAF), was described that specifically promotes growth of Sézary cells in vitro.28–30 However, activity of SAF on fresh leukemic PBL requires the presence of IL-2.

The phenotype of the leukemic population in this case was also unique, as the cells were likely to be of NK origin. The leukemic cells were negative for markers of T (CD3+, CD4+, CD8+, αβ and γδ T cell receptor) and B (Igα) lymphocytes. Furthermore, the cells acquired LAK activity following in vitro culture with IL-2, a well-described property of both human31,32 and canine33,34 NK cells, and the LAK activity we observed in the leukemic population was consistent with that induced in PBL from normal canines. The cytoplasmic granules present in the dog’s leukemic PBL were also consistent with NK (large granular lymphocyte) cell morphology. Collectively, these features resemble the abnormal leukocytes found in human large granular lymphocyte (LGL) leukemia35,36 that is characterized by lymphocytosis of large lymphocytes with cytoplasmic azurophilic granules, immunophenotypic heterogeneity (including both CD3+CD4+CD8+ and CD3−CD4−CD8− cell types), and cytolysis that is not major histocompatibility-restricted and that is augmented by lectins that stimulate IL-2 secretion.37,38 Some LGL leukemia express the NK-specific marker, CD56 (Leu-19).39 In this case, the lack of reagents that recognize canine NK cells precluded the direct identification of the canine leukemic PBL as NK cells.

The clinical aspects of the affected dog we studied had similarities to IL-2–receptor positive adult T-cell leukemia (ATL) in humans, associated with infection by HTLV-1,40 in that the animal had an aggressive lymphoproliferative malignancy of the skin and a lymphoid leukemic blood profile. Similarly, malignant cells of early phase ATL secrete IL-2 and proliferate in an autocrine manner.56 While a retroviral etiology for canine leukemia has yet to be conclusively demonstrated, experimental evidence has been described suggesting possible existence of canine retroviral pathogens.57–59 We made no effort to identify a retroviral etiology for the cancer in this dog.

Leukemias in the dog are well-described, spontaneous clinical entities72–75 classified similarly to their human counterparts.76 Canine leukemias are less common than canine malignant lymphoma, and they represent less than 10% of hemolymphatic cancer in the dog. A recent study of canine lymphoid leukemia indicated the immunophenotype of the majority of affected dogs was that of T cells (pan T+, CD4+, CD8+) (Dr R. Page, personal communication, November 1994). The majority of cases of canine mycosis fungoides were reported to be CD4+.CD8+ although malignant lymphocytes from four of 19 dogs in that study were CD4−.CD8−.25 Thus, the immunophenotype of canine T cell leukemia and cutaneous lymphoma appear to differ from that seen in humans. Nevertheless, based on the dog we have described, this disease may provide a useful animal model for directed immunotherapy. The ability of canine leukemic cells to bind IL-2 at concentrations potentially achievable in vivo in the dog57 implies targeting of malignant IL-2 receptor-expressing cells with an IL-2 immunotoxin conjugate42 could represent an area of future investigation in dogs with lymphoid cancer. The importance of such a model is underscored by the paucity of established, spontaneous outbred large animal models to develop novel approaches for immunotherapy of human cancer.

In summary, we have described a canine cancer patient with a lymphoproliferative disease demonstrating clinical and immunologic features resembling some types of human leukemia/lymphoma. Coordinate expression of IL-2 receptors and IL-2 by the dog’s leukemic PBL suggest an autocrine growth process in vivo. The morphologic features, immunophenotype markers, and LAK activity these cells demonstrated suggests an NK lineage. These observations are important to identification and development of spontaneous animal models of human cancer associated with abnormalities involving the IL-2/IL-2 receptor pathway.

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Functional interleukin-2 receptors are expressed on natural killer-like leukemic cells from a dog with cutaneous lymphoma

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