Allogeneic hematopoietic cell transplantation in a dog with acute large granular lymphocytic leukemia

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Case Description—A 3-year-old 10-kg (22-lb) neutered male Cavalier King Charles Spaniel was referred because of an episode of acute vomiting and diarrhea.

Clinical Findings—On physical examination, mild splenomegaly and prominent submandibular and popliteal lymph nodes were detected. Complete blood cell count revealed a high WBC count, characterized by a moderate lymphocytosis with 62% unclassified cells and severe thrombocytopenia with macroplatelets. On cytologic evaluation, the unclassified cells were described as large, neoplastic lymphoid cells containing a large nucleus with lacy chromatin and a large amount of blue vacuolated cytoplasm containing sparse, very fine azurophilic granules. A diagnosis of acute large granular lymphocytic leukemia of splenic origin was made.

Treatment and Outcome—Following induction chemotherapy, the affected dog underwent allogeneic hematopoietic cell transplantation with dog leukocyte antigen–matched CD34+ cells harvested from a sibling of the same litter. Chimerism analysis revealed full donor engraftment within 2 weeks after transplantation that remained stable for at least 2 years, with the dog remaining apparently healthy at home.

Clinical Relevance—Acute leukemias in dogs are rapidly fatal diseases. If an appropriate donor can be located, allogeneic hematopoietic cell transplantation may offer a feasible treatment, although peripheral blood CD34+ cell harvesting requires the availability of cell separator machines and management of graft-versus-host disease with immunosuppressive agents. (J Am Vet Med Assoc 2015;246:994–997)

Abbreviations

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<tr>
<th>ALL</th>
<th>Acute lymphoblastic leukemia</th>
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<td>DLA</td>
<td>Dog leukocyte antigen</td>
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| HCT | Hematopoietic cell transplan-
| LGL | Large granular lymphocytic |
| TBI | Total body irradiation      |

A 3-year-old 10-kg (22-lb) neutered male Cavalier King Charles Spaniel was evaluated by the referring veterinarian for an episode of acute vomiting and diarrhea. Abdominal palpation elicited signs of pain. No abnormalities were found on rectal examination.

Test results to detect an increase in pancreatic lipase activity, heartworm infection, Lyme disease, ehrlichiosis, and Anaplasma phagocytophilum infection were negative. Results of serum biochemical analysis were within reference range, including electrolyte concentrations. Complete blood count revealed a high WBC count (24,200 WBCs/µL; reference range, 36% to 60%). On cytologic evaluation of a blood smear, the unclassified cells were described as large, neoplastic lymphoid cells containing a large nucleus with lacy chromatin and a large amount of blue vacuolated cytoplasm containing sparse, very fine azurophilic granules.

On physical examination, mild splenomegaly and prominent submandibular and popliteal lymph nodes were detected. Thoracic radiography did not reveal any abnormalities, whereas abdominal ultrasonography revealed mild splenomegaly and mild abdominal lymphadenopathy. A bone marrow aspirate was submitted for cyto-

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logic evaluation, and a blood sample was submitted for flow cytometric analysis. Because of the strong clinical suspicion of ALL, induction chemotherapy including vincristine (0.5 mg/m², IV), l-asparaginase (10,000 U/kg [4,545.5 U/lb], SC), and prednisone (20 mg/m², PO, q 24 h) was administered.

Cytologic evaluation of a bone marrow aspirate revealed complete and orderly maturation of myeloid and erythroid lines (myeloid to erythroid cell ratio, 3:1), although a diagnosis of erythroid hypoplasia was made because of the concurrent anemia. The bone marrow lymphocytes appeared morphologically similar to those seen in the blood, although the number of lymphocytes appeared consistent with the amount of blood present. Large numbers of mature megakaryocytes were noted.

Peripheral blood flow cytometry (performed at Colorado State University Clinical Immunology Laboratory) revealed a homogeneous expansion of CD3+ cells (4,501 cells/µL; reference range, 1,300 to 2,200 cells/µL) composed primarily of CD8+ (3,630 cells/µL; reference range, 450 to 1,000 cells/µL) lymphoblasts that were CD34+. Mature neutrophilia (14,853 cells/µL; no reference range given) was also noted. Considering that bone marrow involvement was minimal, the most likely diagnosis was acute LGL leukemia of splenic origin, in keeping with the moderate anemia, severe thrombocytopenia, neutrophilia, homogenous expansion of CD3+ CD8+ CD34− lymphoblasts, and lack of severe lymphadenopathy.1 A less likely differential diagnosis was stage V LGL lymphoma; this diagnosis was less likely because of the lack of severe lymphadenopathy and the clinical signs of the dog at the time of hospital admission.

The owner elected to pursue allogeneic HCT at North Carolina State University; therefore, the induction phase of chemotherapy, which included cyclophosphamide (350 mg/m², IV) on week 1, vincristine (0.7 mg/m²) on week 2, and adriamycin (30 mg/m², IV) on week 3, was initiated. To identify a suitable DLA-matched donor, 4 related siblings as well as the bitch and sire were located, and a 2-ml blood sample from each dog was sent to the Fred Hutchinson Cancer Research Center for variable-number tandem repeat analysis6 and DLA typing, which was accomplished by modification of a previously described method.3 The combinations of alleles were resolved with in-house custom-built software. The intron-exon boundaries were identified by alignment with DLA-88 genomic sequence or exon 2 sequences of DRB-1. Dog leukocyte antigen typing identified a 14.3-kg (31.46-lb) neutered male sibling as a suitable allogeneic donor.

Both dogs were evaluated at the North Carolina State University Canine Bone Marrow Transplant Unit approximately 5 weeks after diagnosis. At this time, the recipient dog was bright, alert, and hydrated. No abnormalities were detected on physical examination, and serum biochemical analysis results were within reference range. A CBC revealed continuing thrombocytopenia (89,000 platelets/µL; reference range, 190,000 to 468,000 platelets/µL), although the lymphocytosis had resolved. For the donor dog, no abnormalities were detected on physical examination, and results of CBC and serum biochemical analysis were within reference range.

The donor dog had 125 mL of blood collected (replaced by 125 mL of blood obtained from the previous description).6 The donor dog, which had been receiving doxycycline (5 mg/kg [2.27 mg/lb], PO, q 12 h) for 1 week to treat any occult tick-borne diseases, continued to receive doxycycline with concurrent recombiant human granulocyte-colony stimulating factor for mobilization of hematopoietic stem cells for 5 days as previously described.6 Following recombiant human granulocyte-colony stimulating factor administration, parameters of a CBC of the donor dog were high (WBC count, 49,839 WBCs/µL [reference range, 4,390 to 11,610 cells/µL]; neutrophil count, 34,881 neutrophils/µL [reference range, 2,841 to 9,112 neutrophils/µL]; lymphocyte count, 4,893 lymphocytes/µL [reference range, 594 to 3,305 lymphocytes/µL]; monocyte count, 2,492 monocytes/µL [reference range, 75 to 850 monocytes/µL]).

Mononuclear cell apheresis as previously described6 proceeded uneventfully. Over 5 hours, 2.5 X 10^7 CD34+ cells/kg (1.13 X 10^7 CD34+ cells/lb) was collected from the donor dog, which was well above the target dose of 5 X 10^6 CD34+ cells/kg (2.27 X 10^6 CD34+ cells/lb).7 Three aliquots containing 1 X 10^7 CD34+ cells/kg (0.45 X 10^7 CD34+ cells/lb) were cryopreserved at −80°C for infusions of donor lymphocytes in the event of relapse.9,10 The remainder of the harvest product was also cryopreserved after removal and refrigeration of an aliquot containing 5 X 10^7 CD34+ cells/kg for infusion into the recipient the following day after TBI.

After beginning treatment with polymyxin B (5,000 U/kg [17,877.7 U/lb], PO, q 8 h), neomycin sulfate (6 mg/kg [2.73 mg/lb], PO, q 8 h), and doxycycline (5 mg/kg [2.27 mg/lb], SC, q 24 h) for 1 day, the recipient dog received 8 Gy of TBI divided into two 4-Gy fractions separated by at least 3 hours. Immediately after TBI, 5 X 10^7 donor CD34+ cells/kg were infused into the recipient dog over 30 minutes. Posttransplantation care was essentially as previously described,6 except that cyclosporine concentrations were monitored weekly with dosage adjustments made as needed. In addition, blood samples were collected weekly for chimerism analysis.11 Grade IV neutropenia, thrombocytopenia, and anemia were observed as previously described,6,12 although the neutropenia and anemia resolved by the time of discharge from the hospital (although the platelet count was still low; 27,000 cells/µL). The dog was discharged from the hospital 26 days after transplantation with a 5-day course of cyclosporine.

Approximately 15 days after the cessation of cyclosporine (46 days after HCT), the dog developed a mild erythematous rash affecting the ventral aspect of the abdomen, suggestive of acute graft-versus-host disease,13,14 although an increase of liver enzyme activities was not seen. The lesions promptly resolved within 1 week with no medical intervention. Chimerism analysis at regular intervals after HCT indicated the dog progressed to full donor chimerism approximately 2 weeks after HCT, which was maintained since engraftment. The dog remained apparently healthy at home with no further signs of graft-versus-host disease approximately 2 years after allogeneic transplantation.
Discussion

On the basis of recent literature documenting the usefulness of cell separator machines designed for human patients in veterinary species6,13–17 and advances in DLA typing,28 allogeneic HCT is a realistic treatment option for dogs with acute leukemia. To our knowledge, this is the first report in the veterinary literature documenting the use of this procedure for the treatment of a client-owned dog with acute LGL leukemia.

The treatment of choice for acute lymphocytic leukemia in dogs typically involves a cyclophosphamide-, adriamycin-, vincristine-, and prednisone-based chemotherapy protocol (often referred to as CHOP), although there are no reports documenting the efficacy of any one protocol over the other. Regardless, the responses and durability of most protocols are generally disappointing, with most affected dogs dying of progressive disease within weeks after treatment initiation.24 The standard of care in adult humans with ALL that have a combination of poor risk factors (age > 60 years, leukemia count > 30,000 cells/μL, non–T-cell phenotype, poor performance status, Philadelphia chromosome positive at cytogenetic analysis, and lack of mediastinal adenopathy) is allogeneic HCT during the first clinical remission, given that chemotherapy leads to overall disease-free survival in only 35% of these patients.20,21 In addition, considering that relapse of ALL in adults is not curable, allogeneic human leukocyte antigen–identical sibling HCT in second clinical remission is standard of care, with patients having a 35% to 40% chance of long-term disease-free survival.24 More dogs with ALL in first clinical remission need to be treated to determine the efficacy of DLA-matched HCT in this setting.

The purpose of TBI in autologous HCT is to eliminate all residual microscopic disease remaining after systemic chemotherapy and create space in the marrow cavity to allow engraftment of infused CD34+ progenitor cells. Therefore, although a TBI dose of 4.5 Gy is fatal in 50% of exposed people and dogs without aggressive medical care,21 both groups can receive the maximally tolerated dose for normal tissue in fractionated irradiation (approx 10 to 16 Gy) in an effort to increase neoplastic cell death.23 In the allogeneic HCT setting, the prescribed TBI dose is lower (8 to 10 Gy), given that neoplastic cell death is mediated mainly through the development of graft-versus-host disease and subsequent graft-versus-tumor effects.23 For this reason, the transplantation-related mortality rate of patients undergoing allogeneic HCT tends to be much lower than the transplantation-related mortality rate of patients undergoing autologous transplantation, although morbidity and mortality rates following HCT secondary to acute and chronic graft-versus-host disease can be serious. The dog of the present report received cyclosporine in an effort to both dampen graft-versus-host disease and provide suitable recipient immunosuppression to allow engraftment of the donor CD34+ cells.

Dogs have been used in preclinical studies on HCT for humans for many years.23 As such, although most early studies included a small number of client-owned dogs with progressive leukemia treated in a research setting, the notion that leukemia in dogs can be treated by allogeneic HCT is not novel. With advances in DLA typing to select more closely matched donor-recipient pairs, better supportive care, better management of graft-versus-host disease, and use of mobilized CD34+ cells instead of whole bone marrow, we anticipate the use of allogeneic HCT to treat acute leukemias in dogs will provide a considerable clinical benefit over chemotherapy alone.

References

Partial retraction: Allogeneic hematopoietic cell transplantation in a dog with acute large granular lymphocytic leukemia

The article “Allogeneic hematopoietic cell transplantation in a dog with acute large granular lymphocytic leukemia” describes successful (ie, full donor chimerism approximately 2 weeks after transplantation and survival for > 2 years) allogeneic hematopoietic cell transplantation (HCT) in a 3-year-old Cavalier King Charles Spaniel in which a clinical diagnosis of acute large granular lymphocytic (LGL) leukemia had been made. At the time of case management, the authors made a diagnosis of acute LGL leukemia on the basis of clinical signs, initial CBC results (ie, WBC count of 24,200 WBCs/µL, consisting of 23% neutrophils, 12% lymphocytes, 2% monocytes, 1% eosinophils, and 62% unclassified, large, mononuclear cells with lightly vacuolated cytoplasm, a large nucleus, lacy chromatin, and a large indistinct nucleolus), and response to induction chemotherapy.

In retrospect and following a full review of the medical record, it appears that a definitive diagnosis could not be reached in this case. Therefore, the conclusions that this case provides evidence that “allogeneic HCT is a realistic treatment option for dogs with acute leukemia,” that the report documents the first use of allogeneic HCT “for the treatment of a client-owned dog with acute LGL leukemia,” and that the report suggests that “use of allogeneic HCT to treat acute leukemias in dogs will provide a considerable clinical benefit over chemotherapy alone” are retracted by agreement of the editors, authors, and university. The conclusion that allogeneic HCT may be a feasible treatment in dogs remains unchanged.