

Association between cancer chemotherapy and canine distemper virus, canine parvovirus, and rabies virus antibody titers in tumor-bearing dogs

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Objective—To determine the association between cancer chemotherapy and serum canine distemper virus (CDV), canine parvovirus (CPV), and rabies virus antibody titers in tumor-bearing dogs.

Design—Prospective study.

Animals—21 client-owned dogs with various malignancies and 16 client-owned dogs with lymphoma.

Procedure—In study A, serum antibody titers were measured by use of hemagglutination inhibition (CPV titers) or serum neutralization (CDV titers) before and at least 1 month after initiation of chemotherapy. Baseline values were compared with values obtained from a control population of 122 healthy dogs seen for routine revaccination. Titers were considered protective at $\geq 1:96$ for CDV and $\geq 1:80$ for CPV.

In study B, serum IgG titers were measured by use of immunofluorescent assay (CDV and CPV titers) and rapid fluorescent focus inhibition test (RFFIT, rabies titers) at baseline and again at weeks 5, 8, and 24 of a standard chemotherapy protocol for treatment of lymphoma. An IgG titer of $\geq 1:50$ was considered protective for CPV and CDV. An RFFIT titer of ≥ 0.5 U/ml was considered protective for rabies virus.

Results—Significant changes were not detected in CDV, CPV, and rabies virus titers following chemotherapy in tumor-bearing dogs.

Conclusions and Clinical Relevance—Results suggest that established immunity to CDV, CPV, and rabies virus from previous vaccination is not significantly compromised by standard chemotherapy used to treat tumor-bearing dogs. (*J Am Vet Med Assoc* 2001;219:1238–1241)

The effect of chemotherapy on acquired immunity has been studied in human adult and pediatric oncology patients.¹⁻¹¹ Reports^{1,8} indicate that vaccine-induced antibody titers against poliomyelitis, diphtheria,

and tetanus are preserved in children undergoing antineoplastic therapy; however, immunity to varicella, influenza, hepatitis B, and measles is compromised. One early study⁹ of pediatric patients with cancer revealed that patients were susceptible to varicella despite prior chicken pox infection, suggesting that immunity was lost because of disease or treatment. Results of a report by Feldman et al¹⁰ indicated that of 115 previously vaccinated children receiving or having completed chemotherapy for various malignancies, 18% were seronegative for measles antibody, and 8% were seronegative for rubella antibody. Stored serum samples were available for 9 of the seronegative children and revealed that 5 were initially seropositive but became seronegative during or after completion of chemotherapy. In another study,¹¹ effect of chemotherapy on immunity to hepatitis B virus was studied in 49 children undergoing chemotherapy for acute leukemia. Eight of the children were seropositive for hepatitis B antibodies prior to initiation of chemotherapy. All eight became seronegative within 3 months of treatment initiation.

These studies support the hypothesis that chemotherapeutic agents may decrease antibody titers to common viruses. The clinical significance of these findings in veterinary cancer patients is unknown. It has been demonstrated that immunosuppression may result from presence of neoplastic disease itself, particularly lymphoma, or from the use of chemotherapy drugs to treat such disease in dogs.¹²⁻¹⁵ Dogs with lymphoma have impaired cellular immunity, as assessed by in vitro lymphocyte blastogenesis, survival of allogeneic skin grafts, and response to tuberculin challenge exposure after sensitization with Bacille Calmette-Guérin.^{12,13,15} Likewise, impaired humoral immunity has been demonstrated in dogs with lymphoma.^{12,13} Suppressed antibody responses to sheep RBC and to primary and secondary immunization with bacteriophage were documented in dogs with lymphoma in previous studies.^{12,14} Dogs with solid nonhematologic tumors did not have suppression of humoral immunity.¹² Weiden et al¹² reported that baseline serum IgG concentrations for dogs with lymphoma were significantly lower than those of normal dogs, whereas those from dogs with nonhematologic solid tumors did not differ significantly. Similar comparisons were not made during the course of chemotherapy or after treatment. Treatment of dogs with lymphoma with single-agent L-asparaginase or combination protocols using vincristine, cyclophosphamide, and L-asparaginase result-

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ed in impaired humoral (suppressed antibody response to bovine serum albumin and sheep RBC) and cell-mediated (lymphocyte blastogenesis) immune responses in 1 report.¹⁴ To our knowledge, no controlled studies examining the effect of chemotherapy on viral antibody titers have been published in the veterinary literature. Because most viruses of clinical concern (including canine distemper virus and canine parvovirus) are species-specific and because changes in viral titers in response to chemotherapy have varied with each virus examined in human patients, it is impossible to extrapolate this information from the human literature. The practical implications of such information are 2-fold. First, to ensure immunoprophylaxis in veterinary patients undergoing chemotherapy, it is necessary to determine the effect of treatment on preexisting viral antibody titers. Secondly, the public health ramifications of achieving and maintaining protective rabies antibody titers in companion animals are obvious. The decrease in human, canine, and farm animal rabies cases in recent years, despite an increased incidence of wildlife rabies, has been largely attributed to vaccination of dogs.^{16,17} However, the current rabies control measures are based on vaccination status, not viral titer values. Therefore, if a vaccinated animal does not maintain protective titers against rabies while undergoing chemotherapy, the current postexposure guidelines may be inadequate or inappropriate.

We hypothesized that the immunosuppression associated with systemic effects of cancer itself, as well as with the administration of chemotherapeutic agents to tumor-bearing dogs, would result in a decrease in antibody titers to common viruses. In the first phase of the study, we evaluated dogs with various types of cancer to determine whether antibody titers after treatment varied from baseline antibody titers to canine distemper and parvovirus. We also compared initial antibody titers in tumor-bearing dogs to those of healthy dogs seen for routine vaccinations to determine whether their disease state affected their immune status. In the second phase of the study, we performed a prospective clinical trial in which we evaluated dogs with lymphoma that were receiving standardized chemotherapy.

Materials and Methods

Dogs—For study A, 21 client-owned dogs admitted to the University of Missouri-Columbia Veterinary Medical Teaching Hospital (UMVMTH) for chemotherapeutic treatment of various malignancies between July 1998 and April 1999 were included. A previous study group of 122 dogs brought to the UMVMTH for routine revaccination served as the control group. Serum samples from the chemotherapy treatment group were obtained prior to initiation of chemotherapy and again at least 1 month after treatment initiation with a potentially immunosuppressive chemotherapy agent. Blood sampling was part of routine scheduled serum biochemical analyses, and client consent was obtained prior to collection. No treated dogs were vaccinated between pre- and posttreatment sampling times.

For study B, 16 client-owned dogs admitted to the UMVMTH for initial chemotherapeutic treatment of lymphoma between July 1998 and December 1999 were included. Informed owner consent was obtained prior to study

enrollment. Bone marrow aspiration was performed on all dogs before treatment. Serum was obtained prior to chemotherapy and on weeks 5, 8, and 24. A protocol including cyclophosphamide, vincristine, cytosine arabinoside, and prednisone¹⁸ was used for treatment. Dogs with bone marrow involvement (stage V) received L-asparaginase (10,000 to 20,000 U/m², IM) prior to protocol initiation. The only protocol deviations permitted were substitution of chlorambucil (6 mg/m², PO) for cyclophosphamide when hemorrhagic cystitis developed and treatment with L-asparaginase to reinduce remission. No dogs were vaccinated between pre- and post-treatment sampling times.

Serologic assays—For study A, blood samples for both groups were collected, and serum was obtained and stored frozen at -20 C until analyzed. Samples from the control group had been analyzed at the Diagnostic Laboratory, College of Veterinary Medicine at Cornell University as part of a previous study.¹⁹ Samples from the chemotherapy treatment group were analyzed by the same methodology at the University of Missouri Veterinary Medical Diagnostic Laboratory. **Canine distemper virus (CDV)** antibody titers were determined by use of a serum neutralization assay.²⁰ **Canine parvovirus (CPV)** antibody titers were determined by use of a hemagglutination inhibition assay.²¹ Titers were considered protective at $\geq 1:96$ for CDV and $\geq 1:80$ for CPV.¹⁹

For dogs in study B, serum samples were obtained and stored frozen at -20 C until shipped overnight to the Auburn University College of Veterinary Medicine Virology Laboratory for analysis. Canine distemper virus and CPV antibody titers were determined via **immunofluorescent antibody (IFA)** testing. Dilutions of serum were incubated with fixed cell cultures infected with CDV or CPV on 8-well chamber slides. After washing with buffers to remove unbound IgG, a second incubation was performed with a standardized anti-canine-IgG labeled with fluorescein isothiocyanate to detect virus specific anti-viral IgG bound to antigens of either CDV or CPV in the infected cells. Positive and negative controls were compared to patient samples. An IgG titer of 1:50 or greater by an IFA test was considered protective for both CPV and CDV. Rabies virus titers were determined via a **rapid fluorescent focus inhibition test (RFFIT)** performed according to protocol.²² The test is a virus neutralization test in which the endpoint titer is calculated from the highest serum dilution in which there is a 50% reduction in the number of fluorescing foci. An RFFIT titer of 0.5 U/ml or greater was considered protective.

Statistical analyses—In the treated dogs of study A, the possibility of significant increases or decreases in serologic recognition of canine distemper virus and canine parvovirus following chemotherapy was examined, using the sign test. When comparing baseline titers of the treatment group to the control group, the proportions of unprotected dogs were compared by calculating the 95% **confidence interval (CI)** of the difference in the proportions of unprotected dogs between the 2 groups. For all analyses, values of $P \leq 0.05$ were considered significant.

In study B, titers recognizing CDV, CPV, and rabies at 5, 8, and 24 weeks after induction of antineoplastic therapy were compared with pretreatment titers, using a Friedman repeated-measures ANOVA on ranks. The null hypothesis that titer at the time point in question differed from pretreatment titer was rejected when $P < 0.05$.

Results

Study A—Baseline and posttreatment serum samples from 21 dogs were evaluated. Diagnoses included lymphoma (n = 8), mammary carcinoma (2), transitional cell carcinoma of the bladder (4), hemangioper-

icytoma (2), osteosarcoma (2), and 1 each of apocrine gland adenocarcinoma of the anal sac, prostatic carcinoma, and nasal carcinoma. Chemotherapy agents administered included doxorubicin, L-asparaginase (as part of a combination protocol), mitoxantrone, cisplatin, carboplatin, cyclophosphamide, vincristine, prednisone, and cytosine arabinoside. Time between pre- and posttreatment samples ranged from 30 to 272 days (median, 83 days; mean, 117 days). Twelve dogs had pre- and posttreatment samples run together. Nine dogs had additional posttreatment samples tested in a second batch, thus allowing for some intertest variability. Of the 9 dogs with 2 posttreatment titers, the second posttreatment titers were the same as the first in 5 dogs. Three dogs had 2-fold (1-dilution) increases in CPV titers between first and second posttreatment samples, and 1 dog had a return of CDV titer to baseline after a 2-fold decrease detected on initial sampling. Parvovirus titers remained unchanged in 11 of 21 (52%) dogs. Parvovirus titers increased 2-fold in 4 (19%) and 4-fold in 3 (14%) of the dogs tested. Two-fold decreases in CPV titers were evident in 2 (10%) dogs, and a 4-fold decrease was detected in 1 dog. Distemper virus titers remained unchanged in 17 (81%) dogs. One dog had a 2-fold decrease in CDV titer, and 2 (10%) dogs had 4-fold decreases. No significant associations were found between chemotherapy agent, number of days between sampling times, and tumor type with regard to changes in CDV titers. Decreases in CDV titers were not significant.

Serum CPV titers ranged from 1:32 to \geq 1:1,024 (median, 1:256) in the chemotherapy group and from $<$ 1:10 to 1:5,120 (median, 1:320) in the control group. Thirty-three of the 122 (27%; 95% CI, 19.0 to 34.9%) control dogs had less-than-known protective serum CPV titers, compared with 4 of the 21 (19%; 95% CI, 2.0 to 36%) dogs in the chemotherapy group at baseline. This difference was not significant.

Serum CDV antibody titers could not be determined for 5 control dogs because of cell toxicosis. For the remaining 117 control dogs, serum CDV antibody titers ranged from $<$ 1:4 to 1:10,240 (median, 1:256). Baseline serum CDV antibody titers for the chemotherapy group ranged from $<$ 1:8 to \geq 1:256 (median, 1:256). Twenty-five of the 117 (21%; 95% CI, 13.6 to 28.4%) dogs in the CDV control group had less-than-known protective serum CDV antibody titers. Two of the 21 (10%; 95% CI, 3.0 to 23%) chemotherapy group dogs had less-than-known-protective titers. This difference was not significant.

Study B—Baseline and posttreatment CPV, CDV, and rabies virus titers were determined in serum samples from 16 dogs. Tumor staging revealed 4 stage-III, 1 stage-IV, and 11 stage-V cases. All dogs completed the 24-week protocol, but 10 required treatment with L-asparaginase to induce or maintain clinical remission within the first 6 months of treatment. Baseline titers were considered protective (\geq 1:50 using this assay system) for CDV in 10 dogs and inadequate in 6 dogs. After completion of chemotherapy, 5 dogs had titers $<$ 1:50 and 11 dogs had IgG titers \geq 1:50. This change reflected a decrease in CDV titer in 2 dogs, no change

in 2 dogs, and an increase in titer in 3 dogs. Canine parvovirus titers were considered protective (\geq 1:50 by use of this assay system) in 9 dogs prior to chemotherapy and in 6 dogs after chemotherapy. Three dogs had decreases in their titers from 1:50 to 1:10, whereas 6 dogs had low titers that remained low. The RFFIT values for rabies were considered protective if they were $>$ 0.5 U/ml, as they were in 13 of 16 dogs prior to chemotherapy. Rabies virus titers decreased to $<$ 0.5 U/ml in 2 dogs, increased to $>$ 0.5 U/ml in 2 dogs, and remained $<$ 0.5 U/ml in 2 dogs. None of the changes in titers against CDV, CPV, or rabies virus observed at 5, 8, and 24 weeks after induction of antineoplastic therapy were significant.

Discussion

The first goal of our study was to determine whether antiviral titers attributable to vaccination or natural exposure decreased in dogs undergoing immunosuppressive chemotherapy for treatment of cancer. No significant difference was found. In fact, in study A, parvovirus titers increased in 7 dogs, suggesting the possibility of humoral response to natural exposure during multiple hospital visits. The finding in 3 dogs in which CPV titers increased 2-fold between first and second posttreatment samples further supports this. For the serologic assays used, 4-fold (2-dilution) or smaller intertest differences in viral antibody titers may be insignificant and may represent testing variability. Intratest differences of 4-fold or greater are, however, unlikely to be attributable to testing variability. Of the 2 dogs with 4-fold decreases in CDV titer, 1 did not have a second posttreatment sample tested, and the other had the same posttreatment CDV titer at 36 and 168 days. Therefore, variability in titers as a result of performing the assay on 2 different days was not considered a reasonable explanation for the decreases. In contrast to CPV titers, no dogs had increases in CDV titers during the study period. This finding suggests that owners were compliant in the request not to vaccinate dogs during the study period and that the natural exposure to CDV in a hospital environment is less likely than exposure to CPV. The overall changes in CDV and CPV titers were not significant. Although chemotherapy is known to induce neutropenia,^{14,23} thereby suppressing innate phagocytic immunity, our data suggested that sufficient humoral immunity persists to permit maintenance of vaccinal titers and, perhaps, response to natural exposure.

The second goal of study A was to determine whether any differences exist in baseline immunity to CPV and CDV between dogs with cancer and those undergoing routine revaccination. One could argue that dogs with cancer are immunosuppressed and likely to have lower baseline antiviral titers than healthy control dogs. In contrast, we found no significant difference in the proportion of dogs considered to have protective antibody titers between the 2 groups. Although a lower proportion of dogs admitted for cancer chemotherapy had lower than the accepted protective CDV and CPV antibody titers than the healthy controls, the differences were not significant. One obvious explanation is that those dogs undergoing

chemotherapy are more likely to have had regular preventive care because of the conscientious nature of their owners. The significant overall finding of the comparison is that, contrary to popular thought, we found no evidence for a suppressed viral pathogen-specific humoral response, either at admission or following chemotherapy, for dogs with cancer.

One flaw of study A was that serum samples were tested at 2 sites. The serum samples for the historical control population were tested several years prior to initiation of the present study, at which time such testing was not available at the University of Missouri-Columbia. For the present study, identical methods were used, but samples were tested at the University of Missouri-Columbia. In retrospect, it would have been of value to run paired samples at each site to confirm that testing site did not affect our results. For study B, serum samples were sent to Auburn University for testing. This decision was made based on availability of rabies RFFIT at the Auburn laboratory and a desire to run all samples for study B under similar conditions with regard to shipping and testing site. Although testing variability between sites could occur, it was never our intent to make direct comparisons between study A and study B dog titers. Therefore, we believe that our conclusions are valid.

In study B, we evaluated dogs with only 1 tumor type and treated with a standard chemotherapy protocol. The goal of study B was to determine whether antiviral titers decreased in dogs undergoing potentially immunosuppressive chemotherapy for lymphoma. Previous reports¹²⁻¹⁵ have indicated that dogs with lymphoma have impairment of both humoral and cell-mediated immune responses. We found that the overall changes in CDV, CPV, and rabies virus titers were not significant. Low or negative titers in a vaccinated or previously exposed dog may or may not correlate with disease susceptibility because of the potential role of cell-mediated immunity (CMI). These studies were designed to determine whether antibodies declined during chemotherapy. If a significant decline had been detected, subsequent evaluation of the effects of prolonged chemotherapy on CMI was planned. In light of our findings, further evaluation appears to be unwarranted.

Data from these studies suggest that in tumor-bearing dogs undergoing standard chemotherapy, sufficient humoral immunity persists to maintain vaccinal titers. Although immune responses to vaccination were not evaluated in dogs undergoing chemotherapy, the present data suggest that established immunity from previous vaccination is not significantly compromised by standard chemotherapy with the agents we evaluated. Long-term follow-up and a prospective evaluation of immune response to vaccination are necessary to make further recommendations regarding vaccination protocols for patients undergoing chemotherapy.

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