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COMPARISON OF THE BLASTOGENIC RESPONSE OF PERIPHERAL BLOOD LYMPHOCYTES FROM CANINE PARVOVIRUS-POSITIVE AND -NEGATIVE OUTBRED DOGS

CYNTHIA G. OLSEN, MARY I. STIFF, and RICHARD G. OLSEN

Department of Veterinary Pathobiology, The Ohio State University, Columbus, Ohio, U.S.A.

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ABSTRACT

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Lymphocyte blast transformation assays (LBT) were performed on canine parvovirus (CPV) -positive and -negative mongrel dogs randomly selected from a humane facility. Concanavalin A as well as Phytohemagglutinin P stimulation was depressed (p<0.001) in the group of animals shedding CPV compared to CPV-negative dogs.

INTRODUCTION

A panepidemic of canine gastroenteritis occurred in 1978 and, subsequently, the etiological agent was identified as canine parvovirus (CPV). The virus is reported to be transmitted by the fecal-oral route and is stable in the feces for long periods of time (Appel, M.J.G., 1979). Although dogs of all ages are susceptible to canine parvovirus infection, the mortality rate is highest in puppies (Appel, M.J.G., et al., 1979). Besides possible cardiomegaly, myocarditis, pulmonary congestion, and interstitial pneumonia, an abrogation of the immune system was proposed to occur (Carpenter, J.L., et al., 1980; Krakowka, S., et al., 1982). The purpose of this research was to investigate peripheral blood lymphocyte (PBL) function by mitogen stimulation in dogs shedding canine parvovirus. Experiments with CPV infection in conventionalized germ-free dogs did not produce a debilitating enteritis as observed clinically in pet dogs (McAdaraugh et al., 1982, and Pollock, R.V., et al., 1982). Therefore, studies were done on the PBL function of randomly selected mongrel dogs from an humane facility which had naturally acquired CPV.
METHODS

Animals

Outbred dogs of various ages were obtained from the Franklin County Humane Facility in Columbus, Ohio. The animals were classified as CPV-positive or -negative dogs based on the presence of CPV in the feces assayed for by the hemagglutination test (HA) described by Carmichael et al. (1980).

Lymphocyte Blastogenic Transformation (LBT)

The procedure for the canine LBT was a modification of a method described by Krakowka et al. (1975). Citrated blood (40% by volume) was obtained from the cephalic vein. Ten ml of blood was diluted with 20 ml 1640 RPMI media and underlayered with Ficoll-Hypaque (Pharmacia) and centrifuged at 400 g for 30 min (GLC-2 Sorvall, Newtown, CT). The leukocyte-rich interface was recovered and washed twice in media and finally resuspended in 1640 RPMI media with 25 mM Hepes Buffer (GIBCO, Grand Island, NY), supplemented with 1% L-glutamine and 1% antibiotics (Streptomycin, Penicillin, Mycostatin) at a lymphocyte concentration of 1 x 10^6 cells/ml. Cultures were put in 96-well flat bottom microtiter plates (Costar Westraco, Watertown, MA). Each well included 0.1 ml of cells and 0.1 ml of respective concentrations of mitogens and 5% Fetal calf serum. Tests were done in triplicate. Plates were incubated for 72 hours at 37°C in 5% CO2, with moist air. During the final 18 hrs, 0.5 μCi 3H-Tdr per well (6.7 Ci/mmol, NEN, Boston, MA) was added. Cells were harvested onto glass wool filters on a semi-automated cell harvester (Otto Hiller, Co., Madison, WI).

Reagents

Phytomitogens concanavalin A (Sigma Chemical Co., St. Louis, MO) and phytohemagglutinin-P (Difco Laboratories, Detroit, MI) were diluted as indicated for these studies. ConA, PHA-P and fetal calf serum were used from single lots of material. The fetal calf serum was heat-inactivated and used at a final concentration of 5%.

Statistics

The data are given as the mean ± SD. Student's t test was used to determine significance.

RESULTS

Initially, mitogen titration studies were done on peripheral blood lymphocytes from 5 CPV-negative mongrel dogs to determine optimum ConA and PHA-P mitogen doses. Maximum ConA stimulation of 1 x 10^5 lymphocytes was obtained with 4 μg per
well (Fig. 1). The PHA-P stock diluted to 1:10^5 per well provided maximum stimulation (Fig. 2).

In these phytomitogen studies with both ConA (Fig. 3) and PHA-P (Fig. 4), the data indicates a suppression of LBT in parvovirus-positive dogs compared to negative dogs. Average cpm of the ConA LBT in 10 CPV-negative dogs were 16,238 ± 4138 while 8 CPV-positive dogs demonstrated 1360 ± 1162 cpm. In addition, the PHA-P LBT in 9 CPV-negative dogs produced 7087 ± 2570 cpm whereas 8 CPV-positive animals produced cpm of only 395 ± 148.

![Figure 1](image_url)  
Figure 1. Titration of conA in a canine LBT. Counts per minute minus background counts were averaged ± SD.
Figure 2. Dose titration of Pha-P in a canine LBT. Counts per minute minus background counts were averaged ± SD.

Figure 3. ConA LBT responses in CPV-positive and -negative mongrel dogs.
DISCUSSION

Canine parvovirus infection in outbred animals causes a debilitating enteritis along with a marked depletion and necrosis of lymphoid tissues (Peyer's patches, lymph nodes, spleen and thymus) suggesting immune dysfunction (McAdaraugh, J.P., et al., 1992). Recent evidence suggests that CPV infection predisposes dogs to canine distemper virus disease (Krakowka et al., 1982). Since CPV challenge in conventionalized germ-free dogs does not produce the clinical findings of canine parvovirus infection (Pollock, R.V.J., 1982), we decided to study the immune status of outbred animals shedding CPV.

To evaluate the lymphocyte function in these animals, LBT assays with phytomitogens were performed. The group of CPV-positive dogs showed a depressed lymphocyte function. Since these animals were randomly selected from a humane facility, information on variables such as age of animal, time and phase of infection and amount of inoculum was not available. Despite the fact that these conditions were not consistent from animal to animal, a statistically significant state of immunosuppression exists in the animals shedding virus. Although these animals were not screened for possible concurrent viral infections (i.e., canine distemper), examination by the licensed veterinarian showed that the group of CPV-positive dogs demonstrated obvious clinical signs of parvovirus disease without apparent signs of classical viral and/or bacterial infection. Further documentation and investigation is needed to determine the possible mechanism(s) of this suppression, such as direct interaction.
between lymphocyte and virus, change in cell population dynamics or change in mitogen receptors.

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