Changes in Lymphoproliferation and DTH Responses after Vaccination Immediately before Surgery in Puppies

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ABSTRACT. To clarify the effects of the presurgical vaccination with inactivated canine parvovirus vaccine (CPV) on immunological responses to surgery in puppies, we assessed it by measuring the blastogenic responses of lymphocytes and delayed type hypersensitivity (DTH) responses after laparotomy in the non- and vaccinated puppies. The inhalation anesthetic used was isoflurane or halothane. In post-surgery, the blastogenic responses of lymphocytes in the non-vaccinated puppies decreased, especially, those in these puppies with halothane anesthesia (GOI) did significantly, and the duration of this decrease prolonged more than that in the non-vaccinated puppies with isoflurane anesthesia (GOI). However, the responses in the vaccinated puppies with each anesthesia didn’t almost decrease below the presurgical levels. In GOI groups, the DTH responses in vaccinated puppies increased significantly over those in non-vaccinated ones, but in GOI groups, there were few differences between the DTH responses in the non- and vaccinated puppies. These results suggest that the CPV vaccination immediately before surgery possibly prevents the postsurgical immunosuppression, and that GOI may depress the immunocompetence less than GOI. —KEY WORDS: anesthetic, blastogenic responses of lymphocyte, DTH response, puppy, vaccination immediately before surgery.

A transient period of dysfunction in immune system has been repeatedly described following general anesthesia and surgery [2, 6–8, 11, 12, 14, 15, 17, 20–22, 24, 26–28]. Especially, major surgery, such as laparotomy and thoracotomy, has been shown to suppress immunocompetence severely [2, 6, 11, 21]. These immunosuppression must enhance the risk of postsurgical infections [11, 28]. Thus, a search for an adjuvant therapy to the postsurgical immunosuppression has been intensified in recent years [4, 15, 16, 18, 25].

On the other hand, in small animal practice, canine parvovirus (CPV) infection and canine distemper (CDV) are infectious disease that can be characterized by a high morbidity and a high mortality in puppies between the age of about 6 weeks to 6 months [19]. It is almost impossible to prevent the puppies from contacting with the virulent virus, because CPV is ubiquitous and persists for a long period (about 5 to 7 months) in the environment [19]. Therefore, it is a common procedure in the practice to immunize the puppies with modified live or inactivated vaccines at the time of presentation for surgery.

In previous study, vaccination in puppies without surgery has been shown to increase the blastogenic responses of their lymphocytes after the vaccination [13]. A few studies has reported on the immunological responses to vaccination at the time of surgery [10, 16]. The blastogenic responses of peripheral blood lymphocytes after surgery have fallen markedly, although there has been no significant difference in the serum antibody using CDV vaccine between surgical and non-surgical dogs [10]. However, there has no study that definitely compared immunocompetence of surgical dogs which are vaccinated immediately before surgery with those which are non-vaccinated in same surgical conditions.

In this study, we attempted to clarify the effects of presurgical vaccination using an inactivated CPV vaccine on immunocompetence at laparotomy in puppies.

MATERIALS AND METHODS

We tried to clarify the effects of vaccination immediately before surgery in intact puppies by monitoring the pre- and post-surgical blastogenic responses of lymphocytes and by determining the extent of delayed type hypersensitivity (DTH) in the ear after surgery. We possibly eliminated differences in surgery and maintained the puppies under similar conditions. The anesthetic used was isoflurane or halothane. The former has been often used in recent small animal practice.

Animals: Twenty four mongrel puppies with the age 3–4 months old were used after 1–2 months quarantine. The puppies were equally divided into the following groups: Group A, non-vaccination and surgery using isoflurane, nitrous oxide and oxygen (GOI) anesthesia; Group B, vaccination and surgery with GOI; Group C, non-vaccination and surgery using halothane, nitrous oxide and oxygen (GOF) anesthesia; Group D, vaccination and surgery with GOF. All the puppies were clinically normal and were individually isolated for the duration of the experiment.

Vaccination: The inactivated CPV vaccine used in this study was Dhyvac Parvo® (Lot. F-24203, Duphar B. V., Holland). All dogs received vaccine subcutaneously in immediately before surgery.

Anesthesia and surgery: Each anesthetic and surgery was maintained with 1.0 MAC of each anesthetic agent in oxygen for dogs [9] using an end-tidal gas monitor.
(CAPNOMAC ULTIMA®, DATEXI Instrumentarium, U.S.A.) following all dogs were subcutaneously administered with atropine (0.05 mg/kg b.w.) and endotracheal tubing. Duration of anesthesia and surgery was 2 hr. A simple laparotomy was performed in all puppies using standard procedure with dripping of lactated Ringer’s solutions (10 ml/kg/hr) intravenously. Any drugs were not used except for anesthetic agents. The viscera were handled for several minutes, and the abdominal cavity was washed with warmed physiological saline (20 ml/kg b.w.). Then the wound closed with silk sutures.

Blood sampling: Blood samples were collected on the day before the surgery, and subsequently at 0 (1 hr after induction of anesthesia and 30 min after the surgery), 1, 3, 6, 13, 20, and 27 days. Blood samples were heparinized.

Leukocyte and lymphocyte counts: Total circulating leukocyte counts were determined using a microcell counter (Sysmex F-800®, Toa Denshi Kogyo, Japan) and the absolute differential counts for each sample calculated from a standard 200 cell count performed on a Gimsa-stained blood smear.

Blastogenic responses of lymphocytes: The procedure for determining the blastogenic responses of lymphocytes was a modification of previously described MTT assays [22], which used 3-(4,5-dimethyl-thiazolo-2-yl)-2,5-diphenyl tetrazolium bromid (MTT, Dojido, Japan). Peripheral blood lymphocytes were harvested by Ficol separation (SG: 1.077, ficoll-paque®. Pharmacia LKB Biotechnology, N.J.) and washed twice in phosphate buffered saline (PBS), after which the procedure was repeated with RPMI-1640® (Nissui Pharmaceutical, Japan) containing 1% heat-inactivated fetal calf serum (FCS, ICN Biomedicals Japan, Australia). After the final washing, the suspensions were finally adjusted to a concentration of 5 x 10⁸ cells/ml in the culture medium, which was consisted of RPMI-1640 supplemented with 5% heat-inactivated FCS, pyruvic acid (0.11 g/l), -glutamine (0.3 g/l), 2-mercaptoethanol (0.05 mol/l), penicillin (0.1 g/l), streptomycin (0.1 g/l), gentamycin (0.05 g/l), HEPES buffer (1.0 mol/l), and sodium bicarbonate (0.1 mol/l). The suspension was dispensed in 0.2 ml amounts into 96-well flat-bottomed microtiter plates (FALCON® 3075, Becton Dickson, U.S.A.). Then 0.01 ml of mitogens, which were given appropriate concentration, was added to each well, except for the control. The mitogens used were concentration A (Con A, 50 μg/ml), phytohemagglutinin-p (PHA, 100 μg/ml), and pokeweed mitogen (PWM, 5 μg/ml). Culture was maintained at 37°C under 5% CO₂ in air for 72 hr. At 68 hr after the beginning of culture, 0.01 ml of MTT solution (5 mg/ml in PBS) was added to each well. At 72 hr after the beginning of culture, the supernatant fluid in each well was aspirated and then 0.1 ml of dimethyl sulfoxide was added to each well. The plates were shaken to stir the solution in each well. The colorimetry was measured with a microplate reader (MTP-32, Corona Electoric, Japan). The blastogenic responses of lymphocytes were expressed as a stimulation index (SI), which was defined as the ratio of the intensity of experimental sample to the control. The results in the figures were shown as %SI, which was defined that the SI value on the day before surgery was 100%.

Delayed type hypersensitivity (DTH): The determination of DTH were performed 27 days after the surgery. All dogs were intradermally injected in the ear with 100 μl CPV vaccine for specific immunity (DTH-CPV), with 100 μl PHA for non-specific immunity (DTH-PHA), and with 100 μl physiological saline as a negative control. The DTH response of skin was measured by the skin thickness at the injection site with a thickness gauge (Mitsutoyo, Japan), at 24 and 48 hr after the injection.

Statistical analysis: The results were expressed as mean values ± S.E. The statistical significance of the differences between the sample groups was assessed with Student’s t test or Welch test when appropriate. Differences with p<0.05 were considered as statistically significant.

RESULTS

Changes in leukocyte and lymphocyte counts after vaccination and surgery: The number of leukocytes for each group were shown in Fig. 1. The leukocyte counts didn’t change with significant difference among Groups A, B, C, and D. The number of leukocytes decreased significantly 1 hr after the induction or 30 min after the

Fig. 1. Changes in leukocyte counts. Each value represents the mean ± SE. ●●: Group A; △: Group B; △○: Group C; △●: Group D. *: represents significant differences (p<0.05) compared with -1 day. **: represents significant differences (p<0.001) compared with day -1.
surgery. Then the number of leukocytes on day 1 tended to rise higher than the presurgical value, and to recover on day 6 or 13. The number of lymphocytes for each group were shown in Fig. 2. The ordinate was expressed as the percent to the presurgical value. The lymphocyte counts tended to fall at the time of pre- and post-surgery and to recover on day 1.

Blastogenic responses of lymphocytes after the surgery: The changes in the blastogenic responses of lymphocytes to Con A, PHA, and PWM, for each group were shown in Figs. 3–5. In Group A, compared with the presurgical values, the blastogenic responses of lymphocytes were inhibited 30 min after the surgery, and particularly PHA responses were depressed significantly on that time (the mean %SI ± S.E. = 72.9 ± 4.7). These decline continued for 3 days after surgery. In Group B, compared with the presurgical values, the blastogenic responses of lymphocytes tended to increase 1 hr after the induction of the anesthesia, and returned after the surgery. However, these changes had no significant difference. The postsurgical values didn’t almost decline to the presurgical values. Compared with Group D, the response to Con A in Group C had significant difference on day 1 (the %SI values of Group C and D were 67.8 ± 5.3 and 98.4 ± 9.1, respectively), and the responses to PHA in Group C had significant difference on day 1 (71.4 ± 6.0 and 100.3 ± 7.8) and day 6 (75.2 ± 6.0 and 114.7 ± 12.7).

DTH responses: No responses were inhibited by challenge with physiological saline, and no figures were shown. CPV vaccine and PHA produced strong reactions, and the responses were shown in Fig. 6. Regarding the DTH-PHA responses as non-specific immunity, there was no significant difference among Groups A, B, C, and D. The DTH-PHA 48-hr responses in each group decreased.

![Fig. 2. Changes in leukocyte counts. Each value represents mean ± SE. (•—•): Group A, △—△: Group B, ○—○: Group C, △—△: Group D. **: represents significant differences (p<0.01) compared with day -1.](image1)

![Fig. 3. Changes in blastogenic responses of lymphocytes to Con A. Each value represents the mean %SI ± SE. (•—•): Group A, △—△: Group B, ○—○: Group C, △—△: Group D. **: represents significant differences (p<0.01) compared with day -1. #: represents significant differences (p<0.05) compared with Group D on the same day.](image2)
compared with the 24-hr responses. On the other hand, in the isoflurane anesthesia groups, the DTH-CPV 24-hr and 48-hr responses in Group B (the vaccinated group) were significantly higher than those responses in Group A (the non-vaccinated group). In the halothane anesthesia groups, however, there was little difference between Group C (non-vaccinated group) and D (the vaccinated group).

DISCUSSION

General anesthesia and/or major surgery have repeatedly been shown to suppress immunocompetence [2, 6-8, 11, 12, 14, 15, 17, 20-22, 24, 26-28]. It was described also that the cell-mediated immunity was inhibited according to the trauma and surgery [2, 6, 11, 21], and was already suppressed 1 hr after incision in major surgery [17, 22]. In this study, we found that the blastogenic responses of lymphocytes in the non-vaccinated groups (Groups A and C) after the laparotomy were significantly depressed. These suppression continued for at least 3 or 6 days after the surgery. The number of leukocytes and lymphocytes decreased during the surgery in all groups. These findings were consistent with the previous studies [2, 8, 11, 14, 15, 17, 22]. Recently, several adjuvant therapies by drugs were tried to improve this immunosuppression [4, 15, 16, 18, 25]. In both veterinary and human medicine, levamisole has been used in order to improve the immunomodulating effects [15]. It was also reported that Actomin® must have improved the depressed blastogenic response of lymphocytes after various surgeries [25]. In this study, we used the inactivated CPV vaccine, and investigated how changes were produced on the blastogenic response of lymphocytes after the surgery by using the vaccine. As a results, the responses in the vaccinated groups (Groups B and D) almost kept the presurgical levels and were not so low as those in the non-vaccinated groups (Groups A and C). This suggests that it is possible for the inactivated CPV vaccine to act as an immunomodulator.

On the other hand, comparing between the isoflurane anesthesia group (Group A) and the halothane anesthesia group (Group C), the blastogenic responses of lymphocytes in Group A recovered from the declining response, whereas those in Group C still were depressed significantly on day 6 after the surgery. These differences were considered to show differences between the isoflurane and halothane anesthesia. In addition, the DHT-CPV responses in Group B (the vaccinated, isoflurane group) were significantly stronger than those in Group A (the non-vaccinated,
isoflurane group), but there was little difference between
the DTH-CPV response in Group C (the non-vaccinated,
halothane group) and D (the vaccinated halothane group).
These results suggest that halothane may inhibit cell-
mediated immunity stronger than isoflurane, and this
suggestion agrees with previous in vitro studies [1, 3, 5].
Moreover, it is considered that isoflurane anesthesia scarcely
causes depression of the specific immunity to CPV,
compared with halothane anesthesia. Although we used 1.0
MAC of isoflurane or halothane anesthesia in dogs, this
concentration of the anesthesia was lower than that had used
in small animal practice, and was the minimum
concentration for surgery. Therefore, we consider that the
study of the effects under higher concentration of the
anesthesia is needed.

Considering inactivated CPV vaccine as an immunomod-
ulator, the CPV vaccine can inculcate once for all
immediately before the surgery, whereas the administration
with other immunomodulators, such as levamisole, needs
several times during a period of pre and/or postsurgery.
Besides, being used in combination with the minimum im-
muo-suppressive anesthesia, such as isoflurane, the CPV
vaccine can activate specific immunity to CPV and may
prevent depression of the specific immunity against virulent
CPV after surgery. This suggests that inactivated CPV vac-
cine acts as a superior immunomodulator.

The inactivated vaccine used in this study has the faults
that it is delayed to appear specific immunity and duration
of the immunity is shorter than modified live vaccine.
However, there are the advantages that the inactivated
vaccine never cause subclinical infection and never the risk
of reversed mutation to acquire virulence as the modified
live vaccine [19]. Since the inactivated CPV vaccine has
never caused it, this is an appropriate vaccine for presurgical
vaccination. However, we consider that it is necessary to
investigate the effects of other inactivated vaccines,
modified live vaccines, mixed vaccines, and so forth, on the
immunological responses to major surgery in puppies.

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