

## Immunological Responses to Polyvalent Canine Vaccines in Dogs

Tadashi MIYAMOTO\*, Yasuho TAURA, Satoshi UNE, Makoto YOSHITAKE, Sanenori NAKAMA, and Seiji WATANABE<sup>1)</sup>  
Department of Veterinary Surgery, Faculty of Agriculture, Yamaguchi University, Yamaguchi 753 and <sup>1)</sup>Kyoritsu Shoji Co., Ltd., Chiyoda-ku, Tokyo 102, Japan

**ABSTRACT.** The immunological responses to commercially available polyvalent vaccines in dogs were examined. There was a tendency in decreased lymphocyte counts on day 7 in the puppy and adult dogs. There was a significant increase in the blastogenesis of lymphocytes on day 7 and 21 in puppies, whereas no significant changes were seen in the adult dogs. Delayed type hypersensitivity (DTH) responses to phytohemagglutinin (PHA) and canine parvo-virus (CPV) vaccine monitored 0, 3, 8 weeks after vaccination produced strong reactions, in particular those to CPV vaccine rose significantly after vaccination and maintained the higher responses for at least 2 months. Therefore, it is considered that vaccination is immunomodulative rather than immunosuppressive and that DTH responses to PHA and CPV vaccine are helpful to monitor non-specific and specific immune functions *in vivo*.—**KEY WORDS:** canine, immunological response, vaccination.

*J. Vet. Med. Sci.* 57(2): 347–349, 1995

Although there is a limited studies on cell mediated immunity in dogs receiving canine parvovirus (CPV) vaccination [2, 8], the induction of immunosuppression by CPV has been reported [5, 6]. Little is known about vaccines virus interactions and their potential immunosuppressive effects in dogs [11]. This study was aimed to clarify the immunological effects of commercially available polyvalent vaccination in healthy dogs.

Nonvaccinated 4 beagle and 5 mongrel puppies of 2–3 months old (puppy group) and 5 beagle and 4 mongrel adult dogs ranging from 1 to 5 years old (adult group) were used. They were clinically healthy and individually isolated during the period of this experiment. The vaccine that was a mixture with modified live virus (MLV) CPV, MLV canine distemper virus (CDV), MLV canine adenovirus (CAV) 2 vaccine, MLV canine parainfluenza, and two serovars of inactivated leptospira vaccines (Dohyvax7<sup>®</sup>, Solvay Duphar, Weesp, Holland) were administered subcutaneously. Total circulating leukocyte counts were determined by a micro cell counter (CC-110, Toa Medical Electronics Co., Ltd., Kobe, Japan) and the absolute differential count for lymphocytes in each sample was estimated by a standard 200 cell count performed on a Giemsa-stained blood smear. The procedure for determining the blastogenic responses of lymphocytes was modified from previously described ethidium bromide fluorescence assays [4, 10, 13]. Phytohemagglutinin-P (PHA, Difco Laboratories, Michigan, U.S.A.) was used as a mitogen. The blastogenic responses of lymphocytes were expressed as a stimulation index (SI), which was defined as the ratio of experimental to control intensity. Determination of delayed type hypersensitivity (DTH) were performed at 0, 3, 8 weeks after the first vaccination. All dogs were intradermally inoculated in the ear with 0.1 ml CPV vaccine (DohyvaxP<sup>®</sup>, Solvay Duphar, Weesp, Holland) for specific immunity (DTH-CPV), with 0.1 ml PHA for nonspecific immunity (DTH-PHA), and with 0.1 ml physiological saline as a negative control. At 24 and 48

hr after the inoculation, the DTH response of induration of the skin was measured at the inoculation site with a thickness gauge (Mitsutoyo, Tokyo, Japan) [10, 13]. CPV serum antibody titers were assayed by using the hemagglutination inhibition (HI) test [12]. The HI titer was recorded as the reciprocal of the highest dilution of serum showing complete inhibition of hemagglutination. CDV serum antibody titers were assayed by microneutralization (SN) test [1]. SN tests were performed on Vero cells and the SN titer was calculated as the reciprocal of the highest serum dilution that neutralized viral infection and induced cytopathic effects in 2 of 3 replicate cultures.

The results were expressed as mean values  $\pm$  S.E. The statistical significance of the differences between the means of the sample groups was determined by Student's *t* test.  $P < 0.05$  was considered significant.

There was no significant change in lymphocyte counts after vaccination in both groups compared with day 0, however, lymphocyte counts of both groups tend to decrease on day 7 (data was not shown). In the blastogenic responses of lymphocytes to PHA, the puppy showed a increase in SI values after vaccination. In particular, these values were significantly higher on days 7, and 21 (data was not shown). On the contrary, there was no significant change in SI values after vaccination in the adult group and SI values of adult group on day 14 tend to decrease. However, according to each SI value in the adult group, the dogs that had low SI values on day 0 tended to exhibit increased SI values following vaccination, whereas the dogs that had high SI values on day 0 tended to exhibit decreased SI values following vaccination (data was not shown). Regarding the blastogenic responses of lymphocytes to PHA, the puppy group tend to have lower SI values than the adult group on day 0. No responses were initiated by challenge with physiological saline, whereas CPV vaccine and PHA produced strong reactions (Fig. 1). Regarding DTH-PHA responses, the puppy group showed weaker reactions than the adult group. DTH responses at 0 week and 48-hr responses at 3 weeks in the puppy group were significantly weaker than those of the adult group. In the puppy group, DTH-PHA 24-hr responses at 3 weeks were significantly stronger than 24-hr

\* PRESENT ADDRESS: MIYAMOTO, T., Department of Veterinary Medicine, College of Agriculture, University of Osaka Prefecture, Sakai, Osaka 593, Japan.

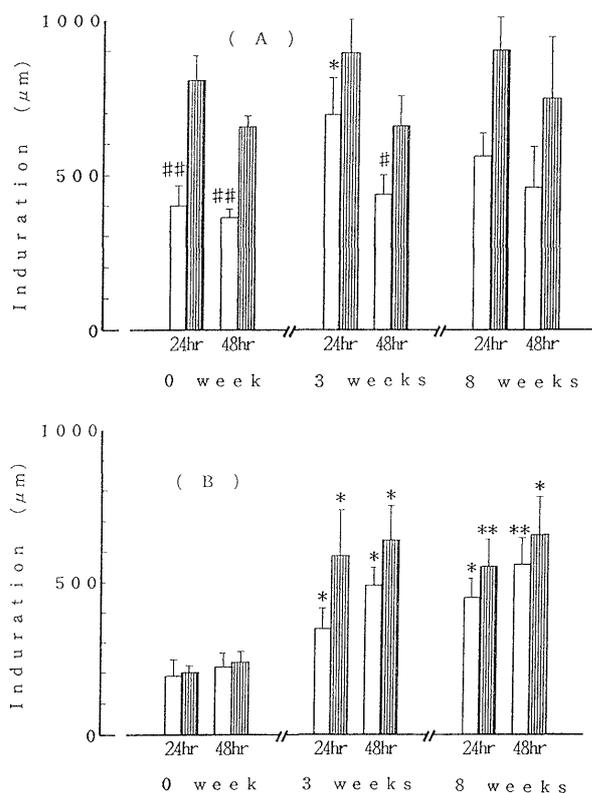


Fig. 1. Changes in delayed type hypersensitivity responses to phytohemagglutinin (A) and canine parvovirus vaccine (B) after vaccination. Each value represents the mean  $\pm$  S.E.  $\square$ : puppy group,  $\blacksquare$ : adult group. Significant differences. \*:  $P < 0.05$ , \*\*:  $P < 0.01$  (compared with the same hr at 0 week), #:  $P < 0.05$ , ##:  $P < 0.01$  (compared with the adult group).

responses at 0 week. DTH-CPV responses at 3 and 8 weeks in both groups were significantly stronger responses at 0 week. DTH-CPV responses in both groups were similar. The changes in CPV and CDV serum antibody titers for each group are shown in Fig. 2. CPV serum antibody titers of each group tend to increase from day 7. Those of the young dogs showed a significant increase on day 28, 35, and 56. Those of the adult dogs showed a significant increase on day 7, 14, 21, 28, 35 and 56. Those of the young dogs rose more slower than those of the adult dogs. CDV serum antibody titers of each group tend to increase after day 14. CDV serum antibody titers rose more slower and lower than CPV serum antibody titers. Those of the young dogs showed a significant increase on day 14, 21, 28, 35 and 56 but those of adult dogs did not show a significant increase.

Several studies have emphasized the induction of immunosuppression by CPV vaccination [2, 3, 7, 9]. However, little has been studied cell mediated immunity after CPV vaccination [2, 8].

In blastogenic responses of lymphocytes to PHA the puppy group had a significant increase on day 7, 21. SI values of the adult group tend to decrease on day 14, however, according to each SI value in the adult group,

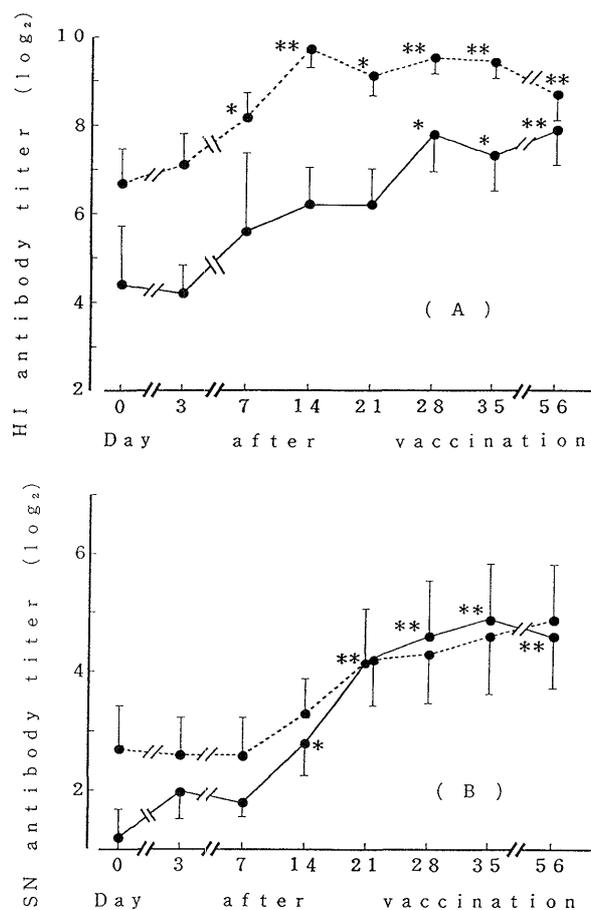


Fig. 2. Changes in canine parvovirus (A) and canine distemper virus (B) serum antibody titers after vaccination. Each value represents the mean  $\pm$  S.E.  $\bullet$ — $\bullet$ : puppy group,  $\bullet$ --- $\bullet$ : adult group. Significant difference. \*:  $P < 0.05$ , \*\*:  $P < 0.01$ .

the dogs that had low SI values on day 0 tended to exhibit increased SI values following vaccination, whereas the dogs that had high SI values on day 0 tended to exhibit decreased SI values following vaccination. The puppy group tend to have lower SI values than the adult group on day 0, therefore, the dogs with higher or lower response in SI values on day 0 tended to show decrease or increase, respectively. These results resembles the one we performed previously with a mixture of inactivated CPV vaccine, MLV CDV and MLV CAV 1 vaccine [10]. Although there was a report that polyvalent commercially available canine vaccines caused significant suppression of lymphocyte response to mitogen [11], it was considered that vaccination might act in an immunomodulative rather than an immunosuppressive fashion. Regarding DTH-PHA responses, the puppy group had a significant increase on 24-hr at 3 weeks and the adult group did not have a significant increase. The changes in DTH-PHA responses resembled those in the blastogenic responses of lymphocytes to PHA. From these results, it was considered that vaccination might not act immunosuppressive, too.

In this study, DTH-CPV responses increased after vaccination and maintained the higher levels for the 2 months' duration of the experiment. Increases in DTH-CPV responses tended to occur according to increases in CPV antibody titers, although there were slight differences between the two responses. These results resemble the one we performed previously [10]. Since DTH-CPV responses can be induced easily and rapidly, it could be used for testing the effects of CPV vaccine in the field, similar to the DTH-tuberculin test.

Transient lymphopenia may occur after vaccination with some MLV-CPV vaccines and this observation has resulted in the ideas that MLV-CPV vaccines are immunosuppressive [2, 5]. In this investigation, both puppies and adult dogs tended to decrease in the number of lymphocytes on day 7, but the decreases were not significant.

CPV serum antibody titers for each group tend to increase from day 7. Those of the young dogs showed a significant increase on day 7, 14, 21, 28, 35 and 56 but those of the young dogs showed a significant increase on day 28, 35 and 56 only. This is, those of the young dogs rose more slower than those of the adult dogs. CDV serum antibody titers of each group tend to increase after day 14. Those of the young dogs showed a significant increase on day 14, 21, 28, 35 and 56 but those of the adult dogs did not show a significant increase. CDV serum antibody titers rose more slower and lower than CPV serum antibody titers. We reported previously that both groups had enough production of CPV and CDV antibodies after the three components of combination vaccines [10]. Although further studies will be needed, it is

possible that vaccine interference is caused by this polyvalent vaccine.

ACKNOWLEDGEMENT. We are indebted to Dr. Arakawa for his critical review of the manuscript.

#### REFERENCES

1. Apple, M. J. G. and Robson, D. S. 1973. *Am. J. Vet. Res.* 34: 1459-1463.
2. Brunner, C. J. and Swango, L. J. 1985. *Compend. Contin. Educ. Pract. Vet.* 7: 979-989.
3. Eugster, A. K. 1980. *Am. J. Vet. Res.* 41: 2020-2024.
4. Ito, Y., Fukamachi, I., Nakajima, K., Kawai, T., and Nakano, Y. 1983. *J. Clin. Exp. Med.* 126: 21-22 (in Japanese).
5. Kesel, M. L. and Neil, D. H. 1983. *Vet. Med. Small Anim. Clin.* 78: 687-691.
6. Krakowka, S., Olsen, R. G., Axthelm, M. K., Rice, J., and Winters, K. 1982. *J. Am. Vet. Med. Assoc.* 180: 137-139.
7. Macartney, L., McCandish, I. A. P., Thompson, H., and Cornwell, H. J. C. 1984. *Vet. Res.* 115: 201-210.
8. Mastro, J. M., Axthelm, M., Krakowka, S., Ladiges, W., and Olsen, R. G. 1986. *Vet. Microbiol.* 12: 201-211.
9. Meunier, P. C., Cooper, B. J., Appel, M. J. G., and Slauson. 1985. *Vet. Pathol.* 22: 60-71.
10. Miyamoto, M., Taura, Y., Une, S., Yoshitake, M., Nakama, S., and Watanabe, S. 1992. *J. Vet. Med. Sci.* 54: 945-950.
11. Phillips, T. R., Jensen, J. L., Rubino, M. J., Yang, W. C., and Schultz, R. D. 1989. *Can. J. Vet. Res.* 53: 154-160.
12. Senda, M., Hirayama, N., Yamamoto, H., and Kurata, K. 1986. *Vet. Microbiol.* 12: 1-6.
13. Une, S., Taura, Y., Nakama, S., and Ejima, H. 1991. *J. Vet. Med. Sci.* 53: 811-816.