Immunogenicity of an Intranasally Administered Modified Live Canine Parvovirus Type 2b Vaccine in Pups with Maternally Derived Antibodies

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The ability of a modified live canine parvovirus type 2b vaccine to elicit active immunization in pups with maternally derived antibodies (MDA) by intranasal administration was evaluated. The vaccine induced seroconversion in 100% of pups with MDA titers of ≥80 and in 51.6% of pups with titers between 160 and 320.

During the early 1970s, a new severe parvovirus-associated disease of pups was observed worldwide (1, 7, 20, 21). The novel parvovirus of canines (canine parvovirus type 2 [CPV-2]) most likely originated by a host species shift from feline parvovirus (FPV) or from FPV-like parvoviruses of wild carnivores. In the 1980s, two antigenic variants of CPV-2 (2a and 2b) arose almost simultaneously and within a few years completely replaced the original variant (6, 14, 15, 23, 26, 27, 28, 29, 34, 35, 36, 37, 38, 40). Intriguingly, only a few amino acid changes in the VP2 of FPV, CPV-2, and CPV-2a and -2b account for important antigenic/biological modifications (18, 25, 30, 39, 41). Additional mutations affecting important residues of the capsid protein VP2 of CPV (residues 297, 300, and 426) have been recognized recently, suggesting that CPV is still evolving (Table 1) (5, 19, 22, 24, 38).

Although the original variant of CPV-2 was completely replaced by the antigenic variants a few years after its appearance, the original CPV type 2 is still used in most commercial vaccines (4, 10, 11, 12). Altogether, there is concern that the antigenic differences between CPV type 2 and the CPV-2a and -2b variants may decrease the effectiveness of the CPV-2-based vaccines (16, 33, 42), and new modified live (ML) vaccines have been developed and licensed using CPV type 2b strains.

A major problem with the immunization of dogs against CPV is the persistence in pups of high levels of maternally derived antibodies (MDA) which may strongly interfere with the development of vaccine-induced immunity. Hemagglutination inhibition (HI) titers of ≥1:20 are able to interfere with an active immune response after vaccine administration, but such titers do not prevent infection with a virulent virus. In contrast, titers of ≥1:80 are considered fully protective against both infection and disease. With such MDA titers, equivalent to 2 to 4 maternal antibody half-lives (about 2 to 5 weeks), pups may fail to be successfully immunized and remain susceptible to infection (9, 12, 31).

In previous studies, both the use of high-titer vaccines, given parenterally, and intranasal vaccination have been suggested as strategies to overcome the obstacle of MDA (Table 2) (2, 3, 8, 17). We are currently investigating whether the use of the new variant CPV-2b as a vaccine may enhance the efficacy of immunization against CPV infection. In a previous study (32), an ML CPV-2b vaccine with a relatively low virus titer administered parenterally proved to be highly effective in overcoming the obstacle of MDA (Table 2). The fact that a low-titer CPV-2b vaccine was successful in inducing active immune responses to CPV in pups with considerable levels of MDA directed our attention to evaluating the immunogenicity of the ML CPV-2b vaccine administered intranasally.

Seventy-eight 5- to 7-week-old pups were used from 16 litters born to bitches that had been vaccinated with a commercially available modified live CPV-2 vaccine before mating. The pups were immunized with a modified live CPV-2b vaccine (strain 29/97), attenuated by 68 passages on Crandell’s feline kidney cells and with a titer of 10^4.5 50% tissue culture infectious doses (TCID50) per ml (32). The vaccine was given by instilling 0.5 ml in each nostril. Pups were vaccinated initially at the age of 5 weeks. If they failed to seroconvert, they were revaccinated when they were 7 weeks old. Blood samples were taken and tested for antibodies to CPV by HI at the time of each vaccination and 15 days after the second dose. Sero logical responses were considered positive if antibody titers had increased at least threefold. The responses to intranasal vaccination are shown in Table 2. The geometric mean of the postvaccinal antibody titers in the pups that seroconverted was 3,564.71 (range, 640 to 20,480).

The main difficulty in controlling CPV infection in pups is due to interfering levels of MDA that can persist up to the age of 12 weeks, or longer, and that can suppress the development of active immune responses to vaccination. Other factors may affect the magnitude of the postvaccinal immune responses to CPV-2 vaccines, i.e., the vaccine virus titer, the degree of virus attenuation (i.e., serial passage level), the antigenic properties of the vaccine strain, and, importantly, the route of administration.

In this study, active immune responses after vaccination were observed in pups with high antibody titers (≥1:80) that...
were expected to block a high percentage of active immune pup responses. A possible explanation for these findings may be either an intrinsic strong immunogenicity of the vaccine used or the antigenic differences between CPV-2 and the variants CPV-2a and CPV-2b. In Table 1, the sequence of vaccine strain 29/97 (68th passage) is shown. Accordingly, the antigenic variants CPV-2a and -2b would be recognized partially by the antibodies raised to type 2 CPV (33), and MDA levels higher than previously estimated would be required to prevent the infection of pups by the CPV variants (9, 12, 13, 14). The interactions between CPV and the canine host were extensively studied in the 1970s and 1980s using exclusively the original CPV type 2. Therefore, the immunological parameters indicative of protection against the 2a and 2b variants of CPV are likely to be reconsidered (13).

Intranasal administration of the ML CPV-2b vaccine proved equally as effective as parenteral administration (Table 2), in spite of an additional 28 serial passages in tissue cultures.

**TABLE 1. Amino acid residues in the VP2 of parvoviruses of the feline subgroup**

| Virus         | Origin, yr | Strain          | Host | Protein at residue |
|---------------|------------|-----------------|------|-------------------|
| FPV           | USA, 1967  | FPV-b           | Cat  | Gly Tyr Asp       |
| MEV           | USA, 1975  | MEV-b           | Mink | Lys Val Asp       |
| CPV-2         | USA, 1978  | CPV-b           | Dog  | Arg Met Asn Ile   |
|               | USA, 1978  | CPV-Norden      | Dog  | Asn Thr Ser Ala   |
|               |            |                 |      | Asp Asn Val Ser   |
| Vaccine A     |            | Cornell 780916  | Dog  |  |
| Vaccine B     |            | 154             | Dog  |  |
| CPV-2a        | USA, 1984  | CPV-15          | Dog  | Leu Thr Gly       |
|               | USA, 1983  | CPV-31          | Dog  | Leu Thr Gly       |
| CPV-2b        | USA, 1984  | CPV-39          | Dog  | Leu Thr Gly       |
|               | USA, 1990  | CPV-133         | Dog  | Leu Thr Ala       |
|               | Vietnam, 2000 | LCPV-V203   | Leop  | Leu Thr Ala Asp Tyr |
|               | Vietnam, 2000 | LCPV-V140   | Leop  | Leu Thr Ala Asp Tyr |
| CPV-2 variants| Vietnam, 2000 | LCPV-2a    |  | |
|               | Italy, 2000 | CPV-616        | Dog  | Leu Thr Pro Gly   |
|               | Italy, 2000 | W42            | Wolf | Leu Thr Pro Gly   |
|               | Italy, 2000 | 136/00         | Dog  | Leu Thr Ala       |
|               | Italy, 2000 | 56/00          | Dog  | Leu Thr Ala       |
|               | Italy, 1997 | 29/97, 68th passage | Dog  | Leu Thr Ala       |

* Dashes indicate identity to the sequence of the CPV-2 strain b (in boldface). CPV-2 variants of recent identification are also reported.

**TABLE 2. Comparison of the HI results of various experiments of vaccination with CPV-2 reported in the literature and the results of this study**

| HI titer | Pratelli et al. (32); CPV-2b strain 29/97, 68th passage; intranasal; titer, 4.5 TCID₅₀ | This paper; CPV-2b strain 29/97, 68th passage; intranasal; titer, 4.5 TCID₅₀ | Buonavoglia et al. (3); CPV-2 strain 17-80, ISS, >60th passage; parenteral; titer, 7.0 TCID₅₀ | Buonavoglia et al. (2); CPV-2 strain 100-115th passage; intranasal; titer, 5.5 TCID₅₀ | Carmichael et al. (11); CPV-2 strain A, 100-115th passage; parenteral; titer, 7.0 TCID₅₀ | Burtonboy et al. (8); CPV-2 strain NL-35D, 37th passage; parenteral; titer, 7.0 TCID₅₀ | Hoare et al. (17); CPV-2 strain NL-35D, 13th passage; parenteral; titer, 7.0 TCID₅₀ |
|----------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------|
| <8       | 1/1 (100)                                                                       | 2/2 (100)                                                                       | NT                                                                              | NT                                                                              | (98)                                                                            | 1/1 (100)                                                                       | 28/29 (96.5)                                                                   |
| 8        | 10                                                                              | 19/20 (95)                                                                      | 16                                                                              | 24/27 (89)                                                                      | 45/45 (100)                                                                    | 16/17 (94.1)                                                                   |
| 16       | 20                                                                              | 8/8 (100)                                                                       | 20                                                                              | 4/4 (100)                                                                       | NT                                                                              | NT                                                                              | (50)                                                                            |
| 32       | 40                                                                              | 12/12 (100)                                                                     | 20/20 (100)                                                                    | 72/78 (92.3)                                                                    | 14/14 (100)                                                                    | 18/22 (82)                                                                      | 31/33 (93.9)                                                                   |
| 64       | 80                                                                              | 10/12 (83)                                                                       | 13/13 (100)                                                                    | 8/11 (72.7)                                                                     | (0)                                                                            | 2/5 (40)                                                                       | 13/13 (100)                                                                   |
| 128      | 160                                                                             | 4/7 (57)                                                                         | 10/15 (66.6)                                                                    | 0/128 (0)                                                                       | 3/17 (17.6)                                                                    | 2/3 (66)                                                                       | 2/3 (66)                                                                       |
| 256      | 320                                                                             | 3/5 (60)                                                                         | 6/16 (37.5)                                                                    | 0/6 (0)                                                                         | 0/1 (0)                                                                       | 6/6 (100)                                                                       | (0)                                                                            |
| 640      | NT                                                                              | 0/4 (0)                                                                          | 0/11 (0)                                                                       | 0/11 (0)                                                                       | 0/1 (0)                                                                       | 6/6 (100)                                                                       | (0)                                                                            |

* Differences in laboratory standardization may slightly affect the results reported in the various experiments.

b The results reported for these studies were obtained in the same laboratory.

c NT, not tested.
Whether the additional cultural passages may have affected the magnitude of the postvaccinal response is difficult to evaluate in this case. However, serial propagation in tissue cells has been shown to affect the vaccine’s ability to overcome the MDA obstacle (Table 2) (8, 17). Also, these findings correlate with previous experiences in which immunization with an ML CPV-2 vaccine was shown to be equally as effective, or more so, when the vaccine was administered by the nasal route, though at a 30-fold-lower vaccinal dose (Table 2) (2, 3).

In conclusion, the findings of this study suggest that good protection against CPV infection may be achieved by the use of an ML CPV-2b vaccine administered intranasally. Active immunization of pups with low-titer ML CPV-2 vaccines has been shown to be affected even by negligible levels of MDA (≥1:20) (Table 2), while our low-titer ML CPV-2b vaccine was able to induce active immune responses in all pups with MDA titers of ≥1:80. The findings of the present study reinforce the proposition that CPV-2b vaccines may confer more adequate protection than CPV-2 vaccines.

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