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Recombinant Vaccine for Canine Parvovirus in Dogs

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VP2 is the major component of canine parvovirus (CPV) capsids. The VP2-coding gene was engineered to be expressed by a recombinant baculovirus under the control of the polyhedrin promoter. A transfer vector that contains the *lacZ* gene under the control of the p10 promoter was used in order to facilitate the selection of recombinants. The expressed VP2 was found to be structurally and immunologically indistinguishable from authentic VP2. The recombinant VP2 shows also the capability to self-assemble, forming viruslike particles similar in size and appearance to CPV virions. These viruslike particles have been used to immunize dogs in different doses and combinations of adjuvants, and the anti-CPV responses have been measured by enzyme-linked immunosorbent assay, monolayer protection assays, and an assay for the inhibition of hemagglutination. A dose of ca. 10 μg of VP2 was able to elicit a good protective response, higher than that obtained with a commercially available, inactivated vaccine. The results indicate that these viruslike particles can be used to protect dogs from CPV infection.

Canine parvovirus (CPV), a member of the autonomous parvoviruses, is the cause of an important disease in dogs. It was first identified in 1978 (1, 4), and it is now endemic around the world. CPV is very related genetically and antigenically to feline panleukopenia virus and mink enteritis virus (5, 14, 15, 18, 24).

Nonenveloped icosahedral capsids of autonomous parvoviruses have a diameter of ≈25 nm and contain a single-stranded DNA of ≈5,000 bases. CPV capsids contain three structural proteins (VP1, VP2, and VP3). VP2 (64 kDa) is an NH_2-terminally truncated form of VP1 (84 kDa) and is the major component of the capsid. VP3 is derived from VP2 by posttranslational proteolytic cleavage and is present only in complete (DNA-containing) virions. Full capsids contain 60 copies of a combination of VP2 and VP3 and contain some VP1 (25). Empty particles do not contain VP3. Trypsin treatment of full particles cleaves VP2 to VP3-like protein (22). There is some evidence that the VP1 terminus is internal and may help neutralize the DNA (25).

Since VP2 in the CPV capsids is far more abundant than the other two components, it is reasonable to think that VP2 alone could be able to self-assemble to make viruslike particles (VLPs). Previously, Mazzara et al. (13) had reported the synthesis of empty capsids using VP1 and VP2 coexpressed in a chimeric papillomavirus expression system. When this paper was in preparation a paper by Brown et al. (3) describing the preparation of VP2 capsids for human parvovirus B19 was published.

In order to get high-reliability and high-level expression of CPV VP2, we have chosen the baculovirus system. Baculovirus has been successfully used to express large amounts of foreign antigens (for a review, see reference 11). A number of VLPs have been obtained for several different viruses, e.g., hepatitis B virus (21), poliovirus (26), bluetongue virus (8), cauliflower mosaic virus (29), and rotavirus (17), among others. The assembly of these particles allows the study of viral morphogenesis and the potential use of these structures as putative vaccines. These vaccines will allow, among other advantages, (i) elimination of viral reservoirs used for the manufacturing of conventional vaccines, (ii) avoidance of new outbreaks due to viral escape during vaccine production, and (iii) facilitation of the eradication of the diseases.

In this paper we report the ability of the CPV coat VP2 protein to form particles by using a recombinant baculovirus. A dose-related study of the immunogenic properties of these particles in dogs, the natural host of CPV, is presented. The potential of these recombinant subunit vaccines is discussed.

MATERIALS AND METHODS

Cells and virus. The *Spodoptera frugiperda* cell line Sf9 (ATCC CRL 1711) was used to propagate wild-type (wt) and recombinant baculoviruses. Sf9 cells were grown in suspension or as monolayer cultures in TNM-FH medium (20) supplemented with 10% fetal calf serum plus antibiotics. The E2 strain of the nuclear polyhedrosis virus of *Autographa californica* (AcNPV) (19), kindly provided by M. Summers (Texas A&M University), was used as the wt virus.

Competent cells of *Escherichia coli* DH5α were used in plasmid DNA transformation according to standard protocols (9).

DNA manipulations. Plasmid pCPV12 containing fragment *Hpa*II-A (≈2.0 kbp) of CPV (10) was used as the parent vector. In the transfer vector pJVP10Z, kindly provided by C. Richardson (National Research Council of Canada), the polyhedrin-coding region has been removed and a unique cloning site, *Nhe*I, has been created (27). This transfer vector also contains a copy of the *lacZ* gene as a reporter under the control of the p10 promoter.

To produce a VP2 fragment with flanking *Xba*I sites, pCPV12 was *Hpa*II digested. A 2.0-kb fragment was isolated on low-melting-point gel agarose and cloned into the *Acc*I sites of pMTL24, yielding pCPV13. VP2 was excised from pCPV13 by digestion with *Xba*I. This fragment was ligated into *Nhe*I-digested, phosphatase-treated pJVP10Z to give transfer vector pCPVEx17. Plasmid DNA was recovered from transformed *E. coli* cells (DH5α) by the alkaline lysis

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