SHORT COMMUNICATION

Raccoon Poxvirus Feline Panleukopenia Virus VP2 Recombinant Protects Cats against FPV Challenge

LIANGBIAO HU,* 1 JOSEPH J. ESPOSITO, † and FRED W. SCOTT* 2

*Cornell Feline Health Center, Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University Ithaca, New York 14853; and †Poxvirus Section, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333

Received October 11, 1995; accepted February 1, 1996

An infectious raccoon poxvirus (RCNV) was used to express the feline panleukopenia virus (FPV) open reading frame VP2. The recombinant, RCNV/FPV, was constructed by homologous recombination with a chimeric plasmid for inserting the expression cassette into the thymidine kinase (TK) locus of RCNV. Expression of the VP2 DNA was regulated by the vaccinia virus late promoter P11. Southern blot and polymerase chain reaction (PCR) analyses confirmed the cassette was in the TK gene of the RCNV genome. An immunofluorescent antibody assay using feline anti-FPV polyclonal serum showed the expressed viral antigen in the cytoplasm of infected cells. Radioimmunoprecipitation with the same antiserum detected a 67-kDa VP2 protein which exactly matched the migration of the authentic FPV VP2 protein by SDS-polyacrylamide gel electrophoresis. Nine five-month-old cats were vaccinated and 21 days later were boosted with the recombinant virus. Peroral FPV challenge 2 weeks after the booster showed that the cats were fully protected as measured by examining clinical signs and total white blood cell counts in peripheral blood. Cats not immunized developed low to very low leukocyte counts following peroral FPV challenge. The nine vaccinated cats showed high FPV neutralization antibody prior to challenge, whereas nonvaccinated cats formed anti-FPV antibodies only after challenge.
Raccoon poxvirus (RCNV) is an orthopoxvirus; two isolates were derived from upper respiratory tissue of two apparently healthy raccoons trapped in Maryland in 1964 (18). A comparative study with several different poxviruses has indicated that RCNV inoculation of cats induces higher autologous VN antibody titer than autologous antibodies induced by other poxviruses tested (19). Laboratory animal studies with a RCNV rabbits virus glycoprotein recombinant (RCN-G) have indicated that oral administration of RCN-G induces rabbits VN antibodies and protection against rabbits challenge in raccoons and in a variety of other animals tested (20). In cats vaccinated perorally, RCN-G has been shown to induce rabbits virus VN antibodies and not spread to cats in contact with vaccinated cats (16). Earlier, it had been shown that the thymidine kinase (TK) gene fragment of RCNV was able to cross-hybridize with that of vaccinia virus (VV), and this enabled construction of RCNV recombinants with various chimeric plasmids designed for foreign gene expression by marker rescue into the VV TK locus (20–22).

In the present study, an infectious RCNV FV VP2 recombinant (RCNV/FPV) was developed by recombining a FPV VP2 expression cassette into the TK locus of the RCNV genome. To generate the recombinant, the FPV VP2 open reading frame (ORF) in 2304 bp excised from a FPV infectious clone was inserted into the plasmid pTKgptF3S (23). The first 33 bases of the ORF were deleted by HincII digestion and the remaining DNA fragment was inserted under control of the VV P11 late promoter in pTKgptF3S. Southern blot analysis followed by sequencing with a CTACTTGCATAGATAGGT primer, designed to anneal 167 bases up from a start codon at the end of the P11 late promoter, enabled selection of an appropriate chimeric plasmid (not shown) for developing recombinant RCNV. Thus, pTKgptF3S containing the left-end of the VV TK followed by TAAAAATATAGTAAATTCTATTTTTTTTTTTATGTTAAAATGAAATCCCTGC-AGGTAACCTGCTGTTGTA and the remainder of VP2 sequences to the stop codon was selected for further analysis (note: nucleotides to the left of the ATG (bold font) are P11 late promoter sequences; nucleotides to the right of the ATG are the in-frame bases of the VP2 ORF from a HincII site in a polylinker sequence that remained after cloning).

Infectious RCNV recombinants carrying the VP2 ORF were obtained by transfecting the chimeric plasmid into CV-1 cell monolayers that had been infected for 2 hr with RCNV by methods described elsewhere (20, 22, 24–26). Rat-2 TK minus cells were then used to select RCNV recombinant viruses by thrice plaque-purifying in the presence of 50 μg 5-bromo-2′-deoxyuridine (BUDR)/ml of medium. Dot-blot hybridizations with a VP2 DNA probe and PCR amplifications of VP2 ORF internal sequences with primers AGTTCAACCAGACGCTGGTGTC (VP2 ORF nucleotides 516–535) and GATCACCAGCTCTGCTGTTGA (VP2 ORF nucleotides 1627–1608) were utilized to identify satisfactory recombinant viral plaques. The VP2 ORF sequences were detected in lysates of virus-infected cells by dot-blot hybridizations, and in recombinant viral genome DNA by detecting PCR amplified VP2 ORF internal sequences in thrice plaque-purified, phenol-chloroform-extracted, recombinant viral genome DNA preparations (data not shown). The recombinant virus isolate replicated in Rat-2 cells under BUDR and formed typical RCNV plaques. The results suggested to us that the inserted VP2 ORF was located in the TK locus of the RCNV genome. Production of the recombinant virus confirmed also that VV TK insertion vectors such as pTKgptF3S can be directly used for foreign gene insertion into the RCNV TK locus (20, 21).

The in vitro expression of VP2 protein by recombinant RCNV/FPV was demonstrated by indirect immunofluorescent antibody (IIFA) and radioimmunoprecipitation (RIP) tests as shown in Figs. 1 and 2. In the IIFA test, RCNV-infected CrFK cells (Fig. 1a) and mock-infected CrFK cells (not shown) did not react with anti-FPV antibodies. FPV-infected CrFK cells showed intranuclear fluorescence (Fig. 1b); however, RCNV/FPV-infected CrFK cell monolayers showed VP2 antigen in the cytoplasm (Fig. 1c). The FPV polyclonal antiserum used in our experiments is a standard diagnostic serum that does not cross-react with RCNV or feline viruses generally tested for in veterinary clinics.

The infection and replication cycle of orthopoxviruses, including raccoon poxvirus, occurs completely in the cytoplasm (27). Consistent with this, the VP2 protein expressed by RCNV/FPV under promoter P11 appears in the cytoplasm during the late infection stage. Interestingly, ADV structural proteins VP1 and VP2 coexpressed in a baculovirus expression system have been shown to be empty particles in the nucleus of baculovirus recombinant-infected insect cells (17). During authentic FPV infection, VP1 and VP2 also appear in the nucleus, mainly in virus particles. Because we expressed only VP2 VP2, we did not examine RCNV/FPV-infected cells for empty FPV particles. The reason why the VP2 transcription product in RCNV/FPV-infected cells is not transported to the nucleus is unclear. However, the marked shut down of host cell protein synthesis by the late time of RCNV/FPV infection and/or lack of other coexpressed FPV components, e.g., VP1, might be responsible.

RIP of [35S]methionine pulse-labeled proteins in lysates of cells infected with RCNV/FPV or RCNV also showed that VP2 protein is expressed by RCNV/FPV and that VP2 is recognized by FPV antibodies (Fig. 2). Four plaque-purified RCNV/FPV recombinant preparations that we made expressed VP2 protein when examined by 8 to 25% gradient gel SDS-PAGE. The expressed protein
migrated at 67 kDa (lanes 1–4), which is the same as reported (9, 10, 13) for mature VP2 protein from FPV-infected cells. The 67-kDa protein did not appear in lysates of cells infected with RCNV (lane 5) or mock-infected cell lysates (not shown).

Specific recognition of VP2 protein by FPV polyclonal antiserum in IIFA and RIP assays suggested to us that the protein expressed by RCNV/FPV is structurally satisfactory for appropriate immunogenic determinants of VP2. Consistent with the data here, in a related study, an FPV VP2 monoclonal antibody identified the same expressed VP2 protein in another RCNV construct that coexpresses FPV VP2 and the rabies virus glycoprotein (Hu et al., unpublished data).

The immune response of nine 5-month-old cats to vaccination with the RCNV/FPV was examined by subcutaneous (SQ) inoculation with the recombinant followed by a booster 21 days later. The vaccinated cats and two unvaccinated cats were challenged by using the USDA standard FPV challenge method (28) on Day 34 after primary vaccination. In Fig. 3 we show that following the initial vaccination with RCN/FPV, the geometric mean serum VN antibody titer of the nine vaccinated cats had reached 1:1,000 by Day 21, the day on which the cats were boosted. Seven days after the booster, the mean serum VN antibody titer was 1:5,000. Thirteen days after booster, the mean VN titer was still 1:5,000; however, by 22 days after the booster the titer was determined to be 1:17,500. No FPV VN antibodies were detected in cats not vaccinated.

Challenge of the vaccinated cats with FPV on Day 13 after the booster did not raise the VN antibody level, suggesting that challenge virus replication was stopped.
by vaccination with RCNV/FPV. On the other hand, unvaccinated animals showed detectable VN titers at Day 5 after FPV challenge. The appearance of circulatory FPV antibodies is consistent with development of a humoral response to infection during challenge. In a separate study (16) with RCNV/FPV at a higher dose in older cats less susceptible to FPV, a single vaccination also showed a significant VN antibody response and protection of cats from FPV challenge.

We have not yet examined the cell-mediated immune response after RCNV/FPV vaccination; however, we speculate that such a response is also playing an active role in providing protection from FPV challenge. In other candidate parvovirus vaccine systems that do not use a recombinant that replicates in the vaccinated animal, e.g., peptide vaccines and baculovirus systems, it is likely that cell-mediated immunity levels are low or not produced.

Total white blood cell and differential counts were measured on Days 0, 3, 5, 9, and 20 following FPV challenge. Fig. 4 shows that the nine vaccinated cats had total white blood cell counts within a normal range of $6.1 - 21.1 \times 10^3/\mu l$ throughout the vaccination and challenge. Also, counts of lymphocytes, monocytes, eosinophils, and basophils remained within normal ranges throughout the experimental course. In contrast, the total white cell counts of cats not vaccinated were less than $5 \times 10^3/\mu l$ on Days 3 and 9 and were $2.2 \times 10^3/\mu l$ on Day 5. Thus, the unvaccinated cats clearly developed panleukopenia, severe lymphopenia, and eosinopenia following FPV challenge. As a typical course of feline panleukopenia, unvaccinated cats had cell counts in the normal range by Day 20 after challenge.

In conclusion, the results in this report show in vivo and in vitro expression of FPV VP2 by a recombinant RCNV/FPV. The recombinant induced a rapid, vigorous, FPV VN antibody response and the response protected cats against FPV challenge administered 13 days after a booster immunization. Of interest, studies on human parvovirus B19 have indicated that B19 virus capsids composed of only VP2 elicit weak neutralizing antibody activity and capsids containing both VP1 and VP2 induce a strong VN antibody activity (30). Here we show that FPV VP2 alone is able to induce sufficient antibodies protecting against FPV challenge.
We strongly suspect that the RCNV/FPV recombinant is safe and efficacious for cats because no untoward effects were apparent during regular examinations of the SQ-vaccinated animals described here. The USDA Standard Requirement for licensure of feline panleukopenia virus vaccines requires that the vaccine stimulates a VN antibody titer of at least 1:8 with at least 20 vaccinated cats and 5 nonvaccinated cats and less than 25% drop from the normal white blood cell count after challenge (USDA Code of Regulations; Ref. 28). Although only 9 vaccinated cats and 2 nonimmunized cats were used in this study, the results here suggested to us that further studies including development of an oral polyvalent vaccine candidate would be warranted.

ACKNOWLEDGMENTS

We thank C. W. Parrish for kindly advising us during these studies and for the infectious clone of FPV. We are grateful to I. Krumina for excellent technical support and to R. Jacobson, J. Casey, and M. Barr for constructive suggestions on this work.

REFERENCES