IMMUNOGENICITY OF PORCINE PARVOVIRUS STRUCTURAL PROTEINS

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Parvoviruses are a family of small DNA containing vertebrate viruses. Parvoviruses are known to infect a wide range of host species including pigs, cattle, dogs, cats, mink, rabbits, mice, and rats (Siegl 1976). Porcine parvovirus (PPV) has been widely documented as a major cause of reproductive failure in swine (Joo, et al 1976, Mengeling and Cutlip 1975). Porcine parvovirus, like other parvoviruses, has an affinity for rapidly dividing cells, infecting and usually killing infected fetuses. High concentrations of PPV virus can be isolated from fetal-infected tissues. Humoral immunity, either as a consequence of natural exposure or from vaccination, prevents viremia and subsequent transplacental transfer (Mengeling et al 1979). Although a number of experimental vaccines have been reported to prevent PPV-induced reproductive failure (Joo and Johnson 1976, Mengeling et al 1979, commercial vaccines have only been recently federally approved in the U.S.A. The efficacy of current commercial vaccines is still under question. Porcine parvovirus, like other parvoviruses, consists of three capsid proteins; A, B, and C, with corresponding molecular weights of 84, 64, and 60,000 daltons (Molitor et al 1982). These proteins have been recently found to contain similar one-dimensional and two-dimensional enzyme digest maps (Molitor et al 1982), suggesting that all three proteins are closely related structurally. The objective of this study was to prepare antisera to PPV polypeptides and intact virus and compare the antibody response generated to the individual polypeptides to that of naturally exposed animal by a battery of serological tests. We wanted to determine which protein(s) were involved in stimulating protective immunity.

Purification of PPV from infected fetuses was as previously described from CsCl gradients (Molitor et al 1982). Virus was disrupted by boiling for 1 minute in buffer containing SDS, and B-mercaptoethanol. Separation of proteins was by SDS-polyacrylamide gel electrophoresis. Proteins were twice gel purified. Approximately 50 µg of each of PPV polypeptides A, B, and C were mixed with 50% Freund's complete adjuvant and injected into rabbits. One rabbit was injected with 50 µg of intact virus plus Freund's adjuvant. Rabbits were boostered at 3 weeks post-inoculation with the same amount of protein and incomplete Freund's adjuvant and again at 5 weeks post-inoculation. Animals were bled and tested for their humoral immune response against PPV by an array of assays including hemagglutination inhibition, agar-gel immunodiffusion (AGID), immunoprecipitation of ^{35}S-methionine-labeled PPV, indirect-fluorescent antibody, immunoauto-radiographic gel analysis following protein blotting and serum neutralization.

A complete line of identity was observed with all antisera in AGID with homogenized PPV-infected fetuses as the antigen. No line was observed with NRS or NPS. All antisera except NPS or NRS immunoprecipitated ^{35}S-methionine labeled, in vitro grown PPV. The density of ^{35}S-methionine labeled intact virus, immunoprecipitated with either A, B, or C, was less than that of either rabbit anti-whole PPV or fetal anti-PPV. Antisera were also tested for reactivity to 48 hour PPV-infected ST cell cultures by indirect fluorescent antibody using fluorescein conjugated to staphylococcus protein A. All PPV antisera stained PPV-infected cells, primarily in the nuclei. No fluorescence was observed from NPS or NRS stained leighton slips. No difference in reactivity of infected cells was detected between the various antisera.

Electrophoretic transfer (Western blot) of proteins to paper and then probing with antisera was a test employed to determine if antisera reacted against dissociated, primary structure polypeptide determinants. Indeed they did, antisera prepared against A, B, and C polypeptides reacted with all three polypeptides as did rabbit anti-PPV prepared against intact virus and pig anti-PPV. Normal rabbit sera or NPS did not react to any of the three polypeptides. Even though all sera reacted to each of the three polypeptides, antisera from rabbits injected with SDS-denatured polypeptides appeared to react stronger to SDS-denatured polypeptides than did antisera from animals injected with intact virus. Anti-B and anti-C reacted against a number of minor molecular weight proteins that were absent from other antisera. These other protein species may be degraded fragments from B or C.

It appears that antisera raised against PPV-polypeptides A, B, and C reacts with determinants on polypeptides A, B, and C by a number of serological tests, but the final question is whether these antisera will neutralize infection. The various antisera at either 1:2 or 1:10 dilution were incubated with in vitro propagated PPV and inoculated onto triplecut St cell cultures. Culture fluids were tested for presence of extracellular virus by hemagglutination and cell cultures were stained with a direct fluorescent antibody conjugate to test for presence of intracellular virus. Cells inoculated with virus alone or virus incubated with NPS or NRS showed high titers of extracellular virus and greater than 80% of the monolayer infected. Both fetal pig anti-PPV and rabbit anti-PPV neutralized 100% of the virus at both serum dilutions. Anti-A sera was found to neutralize 100% of virus infection either at a 1:2 or 1:10 dilution, as did rabbit anti-PPV as well as fetal pig anti-PPV. Anti-B or anti-C sera neutralized 100% of virus at a 1:2 dilution but only 50-70% of extracellular or intracellular virus production at a 1:10 dilution. An explanation for the difference in neutralization between anti-A versus anti-B or anti-C is unknown but it may be due to extra neutralizing determinants on polypeptide A. These results suggest that disrupted denatured viral proteins when given in sufficient quantity and mixed with an adjuvant stimulate neutralizing immunity.

Selected references: Joo, H.S. et al 1976, Arch. Virol. 51:123-129. Mengeling, W.L. and B.C. Cutlip, 1975, Am. J. Vet. Res. 36:1173-1177. Mengeling et al 1979, Am. J. Vet. Res. 40:204-207. Molitor et al 1982, J. Virol. Submitted. Siegl, G. The parvoviruses. Virology Monographs Vol. 15, 1976. Ward, P.C. and Tattersall, P. eds. The Replication of Mammalian Parvoviruses, Cold Spring Harbor Laboratory, 1978.