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Characterization of VP3 as a neutralizing antigen and a cell attachment protein of infectious pancreatic necrosis virus

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To determine the neutralizing antigen of infectious pancreatic necrosis virus(IPNV), monoclonal antibodies were developed against VR-299 and DRT strains in Balb/c mice. Eleven antibodies were obtained and one antibody directed against the VR-299 strain had virus-neutralizing activity(V24). The V24 antibody exhibited different neutralizing activities against four strains of IPNV including VR-299, VR-876, Oregon and DRT. This suggested that V24 antibody recognized the neutralizing and possibly the serotype-determining epitope.

The monoclonal antibody V24 was shown to be specific to VP3, the minor capsid protein of the virus. This antibody also had binding activity on recombinant VP3 expressed in *E. coli*. Additionally, recombinant VP3 inhibited the neutralizing activity of V24 antibody. These results indicate that the neutralizing antibody recognizes VP3 of IPNV and suggest that VP3 can function as the neutralizing antigen of the virus. However, VP3 could not be shown to be the sole neutralizing antigen of the virus since recombinant VP3 inhibited the neutralizing activity of anti-IPNV antiserum only partially.

To locate the neutralization epitope in VP3 recognized by V24, deletion mutants of VP3 were constructed by recombinant DNA technology. The mutant protein which had lost 19 amino acid residues from the N-terminus of VP3, was shown to be recognized by V24 in ELISA and neutralization inhibition experiments. When 38 amino acid residues were deleted further, VP3 was not recognized by the antibody. This result suggests that the antibody recognizes the region following 20th amino acid residue of VP3 molecule.

The function of VP3 was also examined in the viral infection process. The monoclonal antibody V24 inhibited the cell attachment activity of the virus. Additionally, recombinant VP3 in free form inhibited the viral infectivity. These two results suggest that VP3 can function as a cell attachment protein of the virus. The VP3 protein was

also shown to bind to the surface of CHSE-214 cells. In contrast, VP2, the other structural protein of the virus could not bind to cells. Recombinant VP3 was also able to bind to host cells. This further substantiates that VP3 is a cell attachment protein of IPNV.

The cell attachment of recombinant VP3 was inhibited by the neutralizing antibody V24, but cell-attached VP3 was shown to be recognized by the antibody. This suggests that the cell attachment region and neutralization epitope of the VP3 protein are distinct. This was confirmed by examining the cell attachment activity of VP3 deletion mutants. The cell attachment activity of VP3 was completely eliminated by deletion of N-terminal 19 amino acid residues. This result indicated that the cell attachment region resides in the N-terminus of VP3 and is different from the neutralization epitope recognized by V24 antibody.