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Protective immunogenicity of the G protein of hirame rhabdovirus (HIRRV) in flounder using DNA vaccine

Ji Yeon Seo, Ki-Hong Kim, Sung-Koo Kim, Young-Tae Kim and Tae-Jin Choi
Pukyong National University

Introduction

Antiviral DNA vaccine carrying a gene for a major antigenic viral protein have received considerable attention as a new approach in vaccine development. For fish viruses, effects of DNA vaccine encoding viral G gene of infectious hematopoietic necrosis virus(IHNV) and viral hemorrhagic septicemia virus (VHSV)have been demonstrated previously(Lapatra *et al* ., 2001) Hirame rhabdovirus (HIRRV) causes hemorragic disease on flounder. Despite of the importance of aquaculture industry in Korea, researches on DNA vaccine for flounder is very limited. In this study, we developed DNA vaccines for HIRRV using viral glycoprotein (G) and nucleocapsid (N) protein genes.

Material and Method

Virus propagation and Plasmid construction

The HIRRV was propagated in the RTG-2 cell line. The G and N gene were amplified by RT-PCR and cloned into the EcoR I / Xho I and HindIII/ Kpn I site of plasmid vector, pcDNA 3.1(+) (Clontech, USA), which contain a cytomegalovirus promoter upstream the inserted genes.

Expression of recombinant G protein

Cultured fathed minow (FHM) cells were transfected with purified pcDNA-G using calcium-phosphate and expression of the G protein was confirmed by western blot analysis. The expression of the G protein from injected tissue was assayed by immunocytochemistry.

Detection of Mx mRNA by RT-PCR

Total RNA from DNA injected flounder was extracted from kidney using Trizol

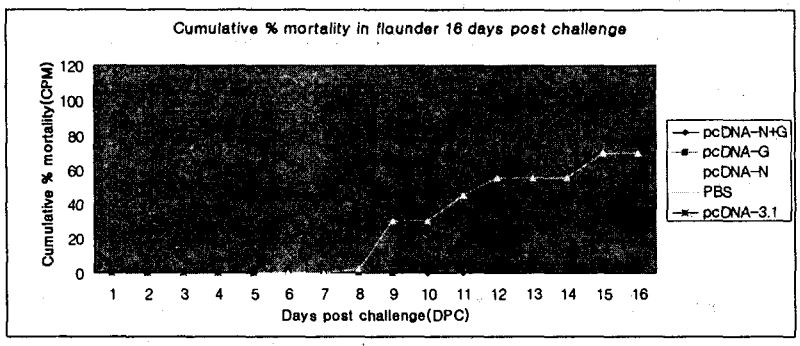
(Gibco BRL., USA). Expression of the Mx gene was confirmed by RT-PCR.

Vaccination and challenge

Flounder with average weight of 2g were injected with 50$\mu\ell$ DNA vaccine containing 5μg of pcDNA-G, pcDNA-N, pcDNA-N+G, or pcDNA3.1(+). At 21 days post-vaccination, groups of fish were challenged by immersion in a inoculum containing 6.7 × 10^4 TCID$_{50}$mℓ^{-1} HIRRV for 90 min at 15℃. The mortality of challenged fish were monitored for 20 days.

Result and conclusion

Hirame rhabdovirus (HIRRV) genes encoding the nucleocapsid protein (N) and the C-terminal two third of the glycoprotein cloned into the expression vector pcDNA 3.1. Western blot analysis showed expression of the target protein in transfected RTG-2 cells. Expression of the target proteins in the injected muscular tissue was confirmed by immunohistochemistry with corresponding antibodies. In addition, higher expression of Mx gene that responsible for non-specific immune responses was detected from the vaccinated fish than non-vaccinated fish. Fish injected with the vector DNA or PBS showed over 95% cumulative mortality 16 day after inoculation. In contrast, fish injected with plasmid containing the N gene, G gene and N+G gene mixture showed 70%, 5%, and 2.5% cumulative mortality, respectively (Fig. 1). These results show that the G gene is effective for inducing protective immunity in the injected fish against HIRRV infection.



<Figure 1> Mortality rate of flounder injected DNA vaccine

Referance

Patra, S. E., Corbeil, S., Jones, G. R., Shewmaker, W. D., Lorenzen, N., Anderson, E. D., Kurath, G. (2001) Protection of rainbow trout against infectious hematopoietic necrosis virus four days after specific or semi-specific DNA vaccination. *Vaccine*, 19, 4001-4019.