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Use of a cDNA microarray to study immunity against viral hemorrhagic septicemia (VHS) in Japanese flounder (*Paralichthys olivaceus*) following DNA vaccination

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Abstract

Japanese flounder, *Paralichthys olivaceus* juveniles were vaccinated against viral hemorrhagic septicemia (VHS) by intramuscular injection of 10 μg of a plasmid DNA vector which encodes the viral hemorrhagic septicemia virus (VHSV) glycoprotein (G) gene under the control of the cytomegalovirus promoter. Experimental challenge of two viral doses (1×10^2 $TCID_{50}$ and 1×10^3 $TCID_{50}$) one month post-vaccination revealed that the G gene was able to induce protective immunity against VHS and this lasted until 21 days after the challenge. The VHSV G-protein gene DNA vaccine had a high protective efficiency, giving relative percentage survival (RPS) values of at least 93%. The defense mechanisms activated by the DNA vaccine were further elucidated by microarray analysis. Non-specific immune response genes such as NK, Kupffer cell receptor, MIP1-α and Mx1 protein gene were observed to be up-regulated by the VHSV G-protein DNA vaccine at 1 and 3 days post-immunization. Also, specific immune-related genes including the CD20 receptor, CD8 alpha chain, CD40 and B lymphocyte cell adhesion molecule were also up-regulated during that time. We observed significant up-regulation of some immune-related genes that are necessary for antiviral defense. Significant up- and/or down-regulation of unknown genes was also observed upon DNA vaccination. Our results confirm previous reports that the VHSV G gene elicits strong humoral and cellular immune responses which may play a pivotal role in protecting the fish during virus infections.

Keywords: Japanese flounder; Viral hemorrhagic septicemia virus (VHSV); DNA vaccine; Microarray analysis; Immune-related gene; Gene expression profiling

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1. Introduction

Viral hemorrhagic septicemia virus (VHSV) is known as one of the most serious viral pathogens of farmed rainbow trout in Europe, causing high mortalities in all ages of fish [1]. The causative agent of VHS is widely distributed among freshwater and marine fishes [1,2]. In 1999, VHSV infection occurred in wild Japanese flounder, *Paralichthys olivaceus*, in Japan during a survey on the distribution of fish viruses in wild marine fishes [3,4].

Severe losses due to VHSV infections have led to many studies on developing vaccines to curb the disease. Among the vaccines that have been tested are killed and attenuated viruses and recombinant proteins [5]. Recently, DNA vaccines against viral hemorrhagic septicemia (VHSV) have been developed for salmonid fish [6–9].

Earlier studies on DNA vaccination against rhabdovirus infection in fish have used the freshwater salmonids as a model. In the present study, we tested the efficiency of DNA vaccination using a marine fish species, the Japanese flounder. The utility of DNA vaccination in fish, especially in rainbow trout, was demonstrated by a protective response against rhabdovirus infections, such as IHNV [10] and VHSV [8], after injection of plasmid DNA containing the G-protein gene of both viruses. It is reported that an early phase of the immune response induced in rainbow trout by DNA vaccination against VHSV and IHNV includes induction of non-specific antiviral defense mechanisms, which is gradually replaced by a more specific immune response [11]. The mechanism of early protection in the vaccinated fish is due to induction of an interferon response. The Mx and MHC Class II genes have been identified as a potential effector mechanism induced by this response [6,9]. However, the effects of DNA vaccination on the kinetics of other immune-related genes have not been elucidated.

Analyses of the expression of a large number of immune-related genes in response to DNA vaccines should lead to a better understanding of the mechanism of immunity in fish. Previously, we conducted EST analyses [12–15] for cloning immune-related genes from Japanese flounder. Very recently, we constructed a cDNA chip that contains approximately 900 different cDNA clones including more than 200 immune-related genes [16]. Microarray analysis can be a useful tool for quantitatively analyzing the expression of some immune-related genes and unknown genes following DNA vaccination. DNA microarrays can measure the expression levels and changes in the expression levels of thousands of genes simultaneously in a particular cell or tissue in a single experiment.

In the present study, we immunized juvenile Japanese flounder with a recombinant plasmid expressing the VHSV G-protein gene. Using a microarray analysis, we studied the elicitation of genes that are responsible for a strong protective non-specific immune response and a specific immune response.

2. Materials and methods

2.1. Cells and virus

VHSV-KRRV 9822, which was isolated from farmed Japanese flounder in Kagawa prefecture in Japan [17], was kindly provided by Dr. T. Isshiki of the Kagawa Prefectural Fisheries Experimental Station. The virus was propagated and titrated in Japanese flounder or the hirame natural embryo (HINAE) cell lines [18].

For virus propagation, HINAE cells were infected with virus and incubated until cytopathic effect (CPE) was observed. The supernatant was collected by centrifugation at 2500 × g for 5 min and stored in 1-ml aliquots at −80 °C until use.