PROTECTION AGAINST *Renibacterium salmoninarum* INFECTION BY DNA-BASED IMMUNIZATION

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Abstract

We report here the results of an experiment in which protection against challenge with *Renibacterium salmoninarum* was conferred by SADI (single antigen DNA-immunization) and ELI (expression library immunization). This is the first report of the potential of DNA-based immunization as an effective method of fish vaccination against bacterial kidney disease.

Introduction

DNA-based immunization refers to the introduction of DNA encoding an antigen into the tissue of an animal in order to elicit an immune response (Tang et al., 1992). DNA-based immunization has some of the advantages of live, attenuated pathogens or live recombinant vaccines without the risk of infection (reviewed in Davis and Whalen, 1995). It has been shown in mammals that immunization with either DNA coding for a single antigen (single antigen DNA-based immunization or SADI) or a eukaryotic expression library of pathogen DNA (expression library immunization or ELI) provides protection against infection by viral, parasitic, or intracellular bacterial pathogens (Davis and Whalen, 1995; Barry et al., 1995).

The intracellular gram-positive pathogen *Renibacterium salmoninarum* is the causative agent of bacterial kidney disease (BKD), a disease that seriously affects wild and farmed stocks of salmonid fishes. Despite many attempts, development of effective vaccines for the prevention of BKD has not been achieved (Newman, 1993). We show here the results of a small scale experiment in which protection against challenge with *R. salmoninarum* was conferred by SADI and ELI. This is the first report of the potential of DNA-based immunization as an effective method of fish vaccination against BKD.

Methods

The vector for the SADI experiment (pGFPp57) was constructed by subcloning the gene coding for the protein p57 from *R. salmoninarum* (Chien et al., 1992) into the expression vector pGFP-C2 (Clontech). The expression libraries for the ELI experiment were constructed by subcloning 500 bp average size fragments of genomic DNA from *R. salmoninarum* and *Aeromonas*

salmonicida into pGFP-C2. These libraries were named RsGFP and AsGFP respectively. Plasmid DNA from pGFPp57, pGFP-C2 and aliquots of the RsGFP and the AsGFP libraries containing approximately 3000 clones each was isolated and purified using Qiagen columns (Qiagen).

Rainbow trout (7-10 cm long) were placed in a recirculating freshwater system and acclimated for two weeks at a temperature of 10°C. Trout were immunized by intramuscular injection of 50 µg/fish of either pGFPp57 or RsGFP DNA. Negative control fish received DNA from pGFP-C2 or the AsGFP library. Ten days after immunization, trout received another injection of 50 µg/fish of the respective plasmid DNA as a boost. Twenty-five days after immunization, trout were challenged by intraperitoneal injection of 0.1 ml of a peptone-saline solution containing $1x10^4$ *R. salmoninarum* cells. The i.p. challenge was repeated 40 days after the first challenge. Mortalities were recorded and dead fish were autopsied to determine the cause of death and for the presence of *R. salmoninarum* in the tissues by Gram-stain and an indirect fluorescent antibody test (IFAT).

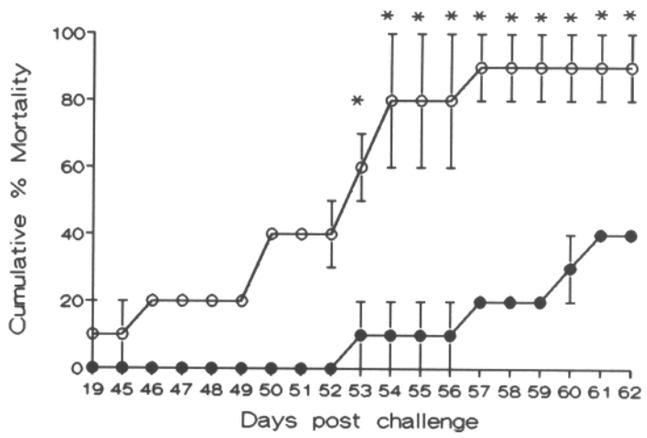

Figure 1: Cumulative percent mortalities of fish immunized with DNA from pGFPC2 (control, open circles) or pGFPp57 (immunized, closed circles) and then challenged with *R. salmoninarum.* * indicates $p<0.01$ (G-test)

Results and discussion

Rainbow trout injected in the muscle with expression vectors containing the DNA coding for p57 (Figure 1) or a random mix of fragments from the *Renibacterium salmoninarum* genome (Figure 2) showed decreased mortality when challenged with *R. salmoninarum* over that of trout injected with expression vectors that lacked *R. salmoninarum* DNA (pGFP-C2 and AsGFP). Protection by the *R. salmoninarum* library was pathogen-specific, since no protection was provided by the *A. salmonicida* library.

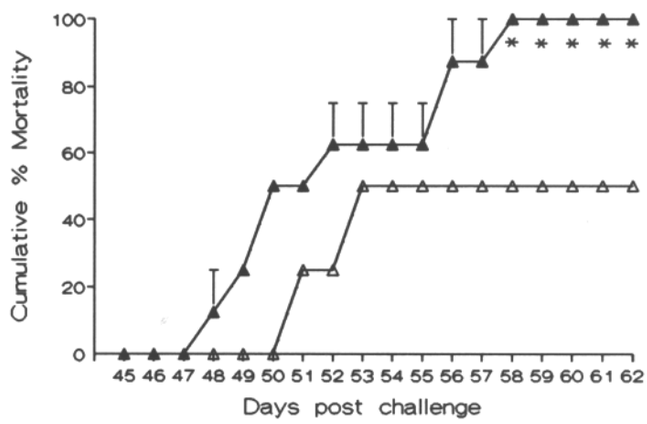

Figure 2: Cumulative percent mortalities of fish immunized with DNA from the libraries AsGFP (control, closed triangles) or RsGFP (immunized, open triangles) and then challenged with *R. salmoninarum.* * indicates $p<0.05$ (G-test).

The levels of protection against BKD achieved by these DNA-based vaccines are striking considering the drastic challenge that was used here. We decided to use challenge by i.p. injection of *R. salmoninarum* because it has the advantage of causing rapid mortalities in contrast to challenge by cohabitation. The protective results that we report here may be an underestimation of the efficacy of these DNA-based vaccines because the infective dose used and the observed rate of infection were more severe than would likely be seen in a natural infection (Murray et al., 1992;

Ellis, 1988). Moreover, i.p. challenge bypasses the natural skin and mucous barriers of the fish that may contribute to protection in a natural infection (Ellis, 1988).

In summary, we have demonstrated here that two different DNA-based vaccines can provide protection against BKD in rainbow trout. Further experiments are underway using larger numbers of fish and challenges by either i.p. injection of the pathogen or by the more natural method of cohabitation (Murray et al., 1992). The protection offered by the *R. salmoninarum* library suggests the presence of genes coding for protective antigens. These genes can be isolated from the library and tested as individual DNA-based vaccines. SADI and ELI may provide cost and time-effective vaccines against BKD and other fish diseases.

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