Mother to fry, successful transfer of immunity against infectious haematopoietic necrosis virus infection in rainbow trout

Syun-ichirou Oshima, Jun-ichiro Hata, Chisako Segawa and Shinya Yamashita

Central Research Laboratory, Nippon Suisan Kaisha Ltd, 559-6 Kitanomachi Hachioji, Tokyo 192, Japan

We have tested whether immunity can be transferred from a mother fish to its fry. Rainbow trout mother fish were inoculated against infectious haematopoietic necrosis virus (IHNV) by intraperitoneal injection of a fragment of the IHNV glycoprotein spanning amino acids 31 to 310. This protein fragment was obtained by isolating the specific cDNA from Japanese IHNV strain HV7601 and expressing it in Escherichia coli. Fry from immunized and control fish were exposed to IHNV at various intervals after hatching, and their mortality monitored. Survival of the fry of immunized fish was significantly greater when exposure to virus occurred 7 days after hatching, and some immunity appeared to persist until at least 25 days after hatching.

Viral infections are a significant problem in fish aquaculture. Although resistance can be conferred by inoculation of individual fish, this is not practical on a large scale. However, recently it has been reported that immunized fertile fish have antibody activity in their ovaries and eggs (Castillo et al., 1993; Avtalion & Mor, 1990), suggesting that immunity might be transferred from mother fish to their fry. If immunity persists in the offspring, then large numbers of fish could be protected by direct inoculation of relatively few mother fish. We have examined this possibility of ‘maternal immunity’ by inoculating rainbow trout against infectious haematopoietic necrosis virus (IHNV) (Ristow et al., 1993) and examining the immunity of their progeny.

IHNV, a member of the family Rhabdoviridae, is a major pathogen of salmonid fish (Amend, 1975). Because of its wide distribution and ability to infect most species of salmonids, IHNV has a severe economic impact on intensively cultured salmon and trout in Europe, North America and Japan. The genome of the virus consists of an unsegmented negative-sense single-stranded RNA of approximately 11 kb that encodes five structural proteins and one nonviral protein (Kurath & Leong, 1985; Kurath et al., 1985; McAllister & Wagner, 1975). A glycoprotein on the surface of IHNV is responsible for eliciting neutralizing antibodies in fish (Engelking & Leong, 1989). Immunity can be induced using either the purified virion glycoprotein, or recombinant glycoprotein produced in E. coli (Gilmore et al., 1988; Xu et al., 1991).

To produce immunity in the fish, we used recombinant DNA procedures to obtain the glycoprotein from IHNV strain HV7601, which is active in Japan (Kasai et al., 1993). Virus stocks were prepared as previously described (Kamei et al., 1987); RNA was isolated from the virus by phenol–chloroform extraction and ethanol precipitation, and cDNA was synthesized with a first strand synthesis kit (Stratagene). IHNV-glycoprotein cDNA was generated by PCR as described previously (Yamada et al., 1993), using oligonucleotide primers based on the sequence reported by Koener et al. (1987) for a North American (NA) strain. The primers GACCAACCCA-CCTGGTCA (sense) and TCTGCTCTCAGAGGCCTT (antisense) produced a product of the expected size, which was cloned into the pSfK vector (Stratagene). The sequence of this fragment is highly homologous to the portion of the NA IHNV glycoprotein commencing at amino acid 31 and extending to amino acid 310 (sequence data accession number D85266). Of the 280 amino acid residues encoded, 16 were found to differ between the two strains.

In order to produce the HV7601 IHNV glycoprotein fragment in E. coli, BamHI sites were incorporated into a pair of primers used to amplify the coding sequence present in the cDNA. The fragment obtained was then subcloned into the glutathione S-transferase (GST) fusion vector pGEX-2T (Pharmacia), and the IHNV glycoprotein over-expressed in E. coli as a GST fusion protein (IHNV-G-GST) as described previously (Yamashita et al., 1992). Because this recombinant protein was insoluble, it was purified to near homogeneity by a conventional inclusion body purification procedure. As a control antigen, GST protein was prepared as described previously.
purified protein (DEM adjuvant emulsified) followed by two
booster shots at 2 week intervals. Blood samples were collected
from each fish by puncture of the caudal vein before, and 7
weeks after, the last injection. The samples were allowed to
clot overnight at 4 °C, then centrifuged at 1500 R, and the
serum stored at -80 °C. Egg samples were homogenized in a
10-fold volume of PBS, centrifuged at 15000 R and the
supernatants stored at -80 °C. The amount of antibodies
against the IHNV-G-GST fusion protein present in these
samples was quantified using an ELISA. IHNV-G-GST fusion
protein was suspended in PBS, and used to coat an ELISA plate
at a concentration of 0.1 μg per well.

The levels of IHNV-G-GST fusion protein immuno-
reactivity in preimmune sera were indistinguishable among the
two groups of fish (Fig. 2a). Following immunization,
booster levels against the IHNV-G-GST fusion protein were
significantly elevated in the sera from both the group A fish
(immunized with IHNV-G-GST) and the group B fish
(immunized with GST) (Fig. 2a). Furthermore, significant levels
of antibodies were also found in egg extracts from the group
A and group B fish (Fig. 2b).

Next, the antisera of immunized fish were tested for their
capacity to block IHNV infectivity in a cultured cell infection
assay similar to that described by Engelking & Leong (1989).
The virus (10^3 p.f.u.) was incubated with equal volumes of a
1/100 dilution of antiserum for 3 h. Triplicate wells in a 96-well
plate (Falcon) containing monolayer cultures of RTG-2 cells
were then incubated with each antiserum–virus mixture. After
3 h, the infected cells were washed and cultured in MEM with
10% fetal calf serum. After 7 days, cell viability was quantified
by MTT [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium
bromide] (Mosmann, 1983). Treatment of IHNV with group A
serum protected over 50% of the cells against IHNV
cytotoxicity, whereas the group B and C sera were without
protective effect (compared to the positive control) (Fig. 2c).
Fig. 2. (a) Immunoreactivity against IHNV-G-GST fusion protein in mother fish sera. Preimmune sera from mother fish and sera obtained 7 weeks post-immunization were characterized by ELISA. Group A mother fish were immunized with IHNV-G-GST fusion protein, group B with GST protein and group C were untreated. Values presented are means from three experiments. (b) Immunoreactivity against IHNV-G-GST fusion protein in fish egg extracts. Eggs were collected from fish 7 weeks after immunization and assayed for antibody against IHNV-G-GST fusion protein by ELISA. Values presented are means from three experiments. (c) Virus neutralizing activities in mother fish sera. The virus neutralizing activities of sera obtained 7 weeks post-immunization were characterized measuring cell viability using MTT. As a negative control, the MTT value of RTG-2 cells without virus infection is presented. As a positive control, the MTT value for RTG-2 cells infected with virus in the absence of serum is presented.

Fig. 3. Survival rate of fry challenged with IHNV strain HV7601. Fifty fry from each female were collected 7 days (a), 25 days (b) and 40 days (c) post-hatching and exposed to approximately 10⁶ p.f.u. of IHNV/ml of water. The graphs show the percentage of fry surviving at daily intervals during 10 days post-infection. Symbols: eggs from group A mother (IHNV-G-GST immunized), IHNV-G1 (△), IHNV-G2 (▲); eggs from group B mother (GST immunized), GST1 (□), GST2 (■); eggs from untreated mother, control 1 (○), control 2 (●).

Thus, treatment with group A serum against IHNV reduced the virus mediated cytotoxicity.

To determine whether fry hatching from the eggs of immune fish had acquired protection against IHNV infection, eggs were collected from each mother fish 3 weeks after the last booster, fertilized, and then kept in running water at 12 °C to hatch. At 7, 25 and 40 days after hatching-out, 50 fry (about 0.4 g each) from each female were challenged for 30 min by exposure to virulent IHNV at a dosage of 10⁶ p.f.u./ml, and their survival was monitored daily for 10 days. Relative to the group B and group C controls, fry from IHNV-G-GST immunized mothers (group A) had clearly increased resistance
at 7 days after hatching, and this resistance appeared to persist to 25 days after hatching (Fig. 3a, b). For example, about 75% of the group A fry challenged 7 days after hatching survived for 10 days, whereas fewer than 50% of the group B and group C fry survived. By 40 days, however, the survival rates among the groups were indistinguishable (Fig. 3c). No difference among groups was observed when fry were challenged 54 days and 72 days after hatching (data not shown). The presence of virus in the dead fry was confirmed by the production of characteristic cytopathic changes in RTG-2 cell cultures (Engelking & Leong, 1989).

The results presented here demonstrate that maternal immunity was successfully transferred to fry, and that the duration of the protective response was maintained for up to 25 days after hatching. As this interval correlates with the period of yolk assimilation under the feeding conditions used, our results raise the possibility that the immunity may depend on the amount of transferred antibody in yolk. If so, then fish producing large eggs, such as the salmonid, may have a particular advantage in conferring ‘maternal immunity’. The transfer of specific antibody to eggs has been reported for plaice (Bly et al., 1986) and tilapia (Avtalion & Mor, 1990), both of whose eggs are rather small. Examination of the degree of immunity transferred to fry in these species would provide a test of the relationship between egg size and the efficiency of ‘maternal immunity’ suggested here.

Vaccination by immersion is a convenient method which is thought to provide adequate protective immunity by the uptake of vaccine through the gills or skin (Amden & Fender, 1976; Johnson et al., 1982). Because the efficiency of uptake greatly depends on the nature of the vaccine, immersion is not a universally applicable method. Vaccination by ‘maternal immunity’ may provide a useful alternative for large scale immunization, and be particularly valuable for protecting fish too young to mount an immune response of their own. Additional studies will be required, however, to determine if immunity can be maintained for an extended period of time. One possibility is that an inoculation schedule different from the one used here, for example, one timed to provide peak immunity during vitellogenesis and oogenesis, will prove to be more efficacious.

To summarize, we have shown for the first time that protection of rainbow trout fry against IHNV infection can be achieved by ‘maternal immunity’. A potential application of this finding is the protection of farmed fish by inoculation of the mother fish.

We are grateful to Dr E. Kawahara for anti-rainbow trout IgM rabbit antibody, Drs M. Brenner, Y. Nishiyama and F. Salati for a critical reading of the manuscript, Dr Y. Nakanisi for helpful discussions.

References


Received 19 December 1995; Accepted 17 June 1996