

EVALUATION OF A SUBUNIT VACCINE TO
INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS

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NUCLEOTIDE SEQUENCE OF A cDNA CLONE ENCODING THE ENTIRE
GLYCOPROTEIN GENE FROM IHNV

Abstract

The nucleotide sequence of the IHNV glycoprotein gene has been determined from a cDNA clone containing the entire coding region. The glycoprotein cDNA clone contained a leader sequence of 48 bases, a coding region of 1524 nucleotides, and 39 bases at the 3' end. The entire cDNA clone contains 1609 nucleotides and encodes a protein of 508 amino acids. The deduced amino acid sequence gave a translated molecular weight of 56,795 daltons. A hydropathicity profile of the deduced amino acid sequence indicated that there were two major hydrophobic domains: one, at the N-terminus, delineating a signal peptide of 18 amino acids and the other, at the C-terminus, delineating the region of the transmembrane. Five possible sites of N-linked glycosylation were identified. Although no nucleic acid homology existed between the IHNV glycoprotein gene and the glycoprotein genes of rabies and VSV, there was significant homology at the amino acid level between all three rhabdovirus glycoproteins.

EXPRESSION IN E.COLI OF cDNA FRAGMENTS
ENCODING IHNV GLYCOPROTEIN GENE

Abstract

Infectious hematopoietic necrosis virus (IHNV), a rhabdovirus, causes a fatal disease in salmonid fish. This agent is endemic to the Pacific Northwest and has endangered the salmon enhancement programs and trout industry there. The development of a safe, easily produced and economical vaccine has been widely sought by the industry. Since the virus is transmissible by water and endemic among wild populations of fish, attenuated or killed vaccines were unsuitable and the development of a subunit vaccine produced by recombinant DNA techniques was sought.

In an effort to develop a subunit vaccine, cDNA clones to each IHN viral mRNA species were made and a physical map of the viral genome was constructed (Kurath and Leong, 1985; Kurath et al., 1985). The virion glycoprotein, G, was identified as the antigen responsible for inducing protective immunity, (Engelking and Leong, in preparation) and a cDNA clone encoding the entire glycoprotein gene was isolated (Feyereisen-Koener et al., in preparation). This gene was digested with the restriction endonuclease, *Sau3A*, and shotgun-cloned into **pATH** expression vectors (Tanese, Roth, and Goff, 1985) to produce trpE-G fusion proteins in *E. coli*.

This report describes the trpE-G gene fusions that resulted in the production of a protein detected with antisera to the IHNV glycoprotein. It also presents preliminary data demonstrating the development of protective immunity to IHNV after exposure to the fusion protein.

INTRODUCTION

Infectious hematopoietic necrosis virus (IHNV) is a rhabdovirus that infects salmon and trout. It produces an acute disease resulting from the destruction of the hematopoietic tissue in the kidneys and may lead to the loss of an entire fish hatchery population (Pilcher and Fryer, 1980). At the present time the only effective means for controlling this disease is the complete destruction of stocks of infected fish and sterilization of hatchery. These control methods are expensive and in some cases lead to the destruction of valuable fish stocks. Thus, an effort was made to develop a vaccine for IHNV.

Previous studies have indicated that protective immunity to IHNV was induced in sockeye salmon (Oncorhynchus nerka, Walbaum) with a strain of IHNV which had been passed on steelhead trout cells in tissue culture for more than 40 times (Tebbit, 1976; Fryer et al., 1976). However, the attenuated strain was not effective as a vaccine in rainbow trout and was actually lethal in young trout (Winton, personal communication). In order to avoid some of the problems attendant with attenuated vaccines the development of a subunit vaccine to IHNV was undertaken.

One of the first considerations in such an undertaking was the identification of the viral protein responsible for the induction of protective immunity in fish. For the rhabdoviruses, vesicular stomatitis (VSV) and rabies, the viral glycoprotein is the only viral antigen which induces neutralizing antibody and protective immunity. We have shown that IHNV glycoprotein is a similar viral antigen (Annual Report, 1984). In the fish system where the immune response is less clearly understood, it was necessary that the IHNV glycoprotein be tested for its immunogenic properties. We report here that purified IHNV glycoprotein from Type 1 IHNV will induce protective immunity in salmonid fry to four other biochemical

types of IHNV (Hsu, Engelking, and Leong, in press). Immune induction occurs after intraperitoneal inoculation or immersion in a solution of purified glycoprotein from Type 1 IHNV and protective immunity is produced in fish as small as 0.5 g and lasts for at least 30 days. It is critical in the development of a subunit vaccine to select a viral antigen that will induce immunity to all variants of a pathogen existing in the environment.

MATERIALS AND METHODS

Cells and virus

The chinook salmon (Oncorhynchus tshawytscha) embryo cells, CHSE-214, were obtained from J. L. Fryer, Oregon State University, Corvallis, Oregon and from the epithelioma papillosum cyprini cells (EPC) were obtained from D. Mulcahy, National Fisheries Research Center, Seattle, Washington. The cells were grown in Eagle's minimum essential medium (MEM) supplemented with fetal calf serum (5%), NaHCO₃ (0.075%), penicillin (100 i.u./ml) and streptomycin (100 ug/ml). The CHSE-214 cells were used to prepared virus stocks and the EPC cells were used for virus assays.

Assays were performed using confluent EPC cell monolayers grown in 96-well tissue culture plates (Falcon). Samples from infected fish were prepared as described (Engelking and Leong, 1981), sterilized by filtration (0.2 um acrodisc, Gelman) and diluted in MEM (without fetal calf serum). Duplicate samples (0.05-0.1 ml) of each dilution were placed on monolayers in individual wells and allowed to adsorb for 60 minutes. Sample inoculum was removed from the wells after adsorption and 1-1.5 ml of MEM growth medium was added to each well.

The Round Butte Type 1 and the Elk River Type 3 strains of IHNV were obtained from W. Groberg, Oregon Department of Fish and Wildlife. The

Hagerman Valley Type 2 strain was obtained from N. Wood, Rangen Research Laboratories, Idaho. The Coleman River National Hatchery Type 4 strain and Cedar River Type 5 strains were obtained from D. Mulcahy, National Fisheries Research Center, Seattle, Washington. The viral glycoprotein was Isolated from the Round Butte strain of IHNV after extensive purification of the virus by isopycnic and velocity sedimentation in an ultracentrifuge. All virus strains were prepared by growing the virus at a multfpliclty of infection of 0.01 to 0.001 **TCID₅₀** per cell on CHSE-214 cells as previously described (Engelking and Leong, 1981).

The virus used for challenges in the immunization trials was prepared from a stock of virus which had undergone no more than three passes in tissue culture after isolation from Infected fish.

Purification of the viral glycoprotein

The IHNV glycoprotein was purified as previously described (Annual Report, 1984). Briefly, purified IHNV was incubated in 1% Triton X-100 to selectively solubilize the G protein. The other viral proteins were removed by centrifugation. The Triton X-100 in the glycoprotein preparation was removed by batch elution with SM-2 beads (BioRad).

Analysis of purified glycoprotein by SDS-polyacrylamide gel electrophoresis

(PAGE).

Electrophoresis was performed in a 10% polyacrylamide gel with a 3% stacker as described by Laemmli (1970). Protein bands were visualized by staining the gel with silver nitrate as described by Allen (1980).

Immunization of fish

The fish utilized in these studies were obtained from Oregon Department of Fish and Wildlife (ODFW) hatcheries through Richard Holt, Oregon State Fish Pathologist. The fry were obtained one week before immunization and

acclimated at the Fish Disease Laboratory in Corvallis, Oregon. The fry were maintained on BioDiet (BioProducts, Warrenton, Oregon) in well water flowing at 0.5 gallons per minute at **12°C**. When the fish were 0.5 g in size they were vaccinated by direct immersion or intraperitoneal inoculation.

The fish were anesthetized with benzocaine and injected intraperitoneally with 10 ul of purified IHNV glycoprotein from a 250 ul glass syringe with a 30 gauge disposable needle. The inoculated fish received approximately 450 ng of purified glycoprotein.

Immersion Immunization was performed on non-anesthetized fish in a beaker containing purified glycoprotein (40-50 ug/ml) in 0.01 M Tris-hydrochloride, pH 7.6. The fish were exposed to the protein solution for 1-2 minutes and then released into a holding tank containing running water at **12°C**.

All immunized and mock immunized fish were maintained in well water flowing at 0.5 gallons per minute at **12°C** for 30-60 days before challenge with live IHNV virus.

Virus challenge

Challenges were made with lots of 25 vaccinated and unvaccinated control fish for each virus dilution (Amend, 1981). The fish were exposed to different virus dilutions in 1 liter of water containing 10^{-2} , 10^{-3} , 10^{-4} , or 10^{-5} dilutions of the virus stock: These dilutions represented approximately 2×10^6 , 2×10^5 , 2×10^4 , and 2×10^3 TCID₅₀ doses per ml of water. The fish were held in the virus-containing water for 18 hours and then returned to holding tanks. Dead fish were removed daily, recorded, weighed, and processed for IHN virus isolation.

Isolation of IHN from infected fish.

Dead fish were processed immediately for virus isolation. The fish were weighed and diluted (w/v) 1:10 with Hanks Buffered Salt Solution, and then

macerated in a stomacher processor (Tekmar). The resulting suspension was clarified by centrifugation and the supernatant solution was treated (1:5 dilution) with antibiotics, Penicillin/Streptomycin (1,000 units/ml, 1,000 ug/ml), Fungazone (500 IU/ml) and Gentamicin (0.25/ml) in PBS overnight at 4°C. The next day the fluid was inoculated directly onto CHSE-214 cells and EPC cells in multi-well plates as previously described (Engelking and Leong, 1981). The cells were observed daily for cytopathic effects for two weeks. For those samples where CPE was questionable, the tissue culture fluid from the sample well was removed and reinoculated onto CHSE-214 cells and subsequently labeled with 35S methionine as described (Hsu et al., 1985).

Only those fish from which IHNV was isolated were considered in these studies. The percentage mortality was determined for each group and the relative percentage survival was calculated for each group of vaccinated compared to controls as follows: (Johnson, Flynn, and Amend, 1982).

$$\text{Relative percentage Survival [RPS]} = 1 - \frac{\% \text{specific loss vaccinated}}{\% \text{specific loss controls}} \times 100$$

Statistical analysis

The data were analyzed by logit regression. The number of fish that died from IHNV infection was taken to be a binomial random variable with the probability of death, p , depending on the various factors under investigation. The model for the dependence of p on the factors was that the logarithm of the odds had a linear regression on the quantities of interest,

$$\ln (p/1 - p) = A + bX + \dots$$

This defines a logit linear model which encompasses the structure of regression and analysis of variance into binomial data (McCullagh and Nelder, 1983). The type of administration of vaccine (inoculated, immersed, or control) and particular experiment were treated as qualitative factors. Both

were found significant in the logit linear model as was the effect of the log dilution of virus.

RESULTS

Challenge of fish with homologous IHN virus strain

Experiments with rainbow trout (*S. gairdneri*) and kokanee fry (*O. nerka*) have previously shown that immunization with purified glycoprotein (G) does elicit a protective response to subsequent IHN infection. The results are summarized in Tables (3 and 4) and Figures (1 and 2). The data is highly significant by statistical analysis. In general more protection is afforded at lower concentrations of virus which is more representative of natural levels that fish will encounter.

Challenge of fish with heterologous IHN virus strains

A critical question in the development of any viral vaccine is whether cross immunity will arise from vaccination with a vaccine derived from a single virus strain. To answer this question fish were immunized with glycoprotein (G) isolated from Type 1 IHN Round Butte and challenged with various isolates representing the other four biochemical types (Table 1). The specific biochemical differences in N and G proteins which have given rise to this typing is shown in Figure 7. The molecular weight values for the varying proteins are listed in Table 2.

Type 2 IHN challenge

A Hagerman Valley, Idaho IHN isolate was chosen to represent the Type 2 strains. This area represents a potential source of infection to the Columbia River basin. Fish were immunized with glycoprotein from Round Butte IHN (Type 1) and challenged with Hagerman Valley IHN (Type 2). The experimental results are shown in Table (5). Although the results are somewhat variable statistical analysis confirms that protection is afforded. A second trial with Hagerman Valley IHN (Type 2) gave more consistent results although only three viral dilutions were used as seen in Table (6) and Fig. (3).

Statistically these results are significant and show that the immunized fish are protected.

Type 3 IHNV challenge

An Elk River, Oregon IHNV isolate was chosen to represent the Type 3 IHNV strain. This strain has been found at the Dworshak Hatchery and may be important in the upper Columbia River region. Again rainbow trout were immunized with the Type 1 purified glycoprotein and challenged with the Elk River Type 3 IHNV isolate. As seen in Table (7) and Figure (4) immunization resulted in dramatic difference in fish survival to virus challenge. Protection levels approximate that of the homologous Type 1 IHNV challenges.

Type 4 IHNV challenge

The type 4 IHNV isolates all are temperature sensitive variants from the Coleman Hatchery (Hsu, Engelking, and Leong, in press; Mulcahy, Pascho, and Jenks, 1984). A biologically characterized isolate from 1980 was used to challenge Type 1 glycoprotein immunized rainbow trout. Good protection to the heterologous challenge was afforded by the vaccination (Table 8, Figure 5). Immersed and injection routes of immunization were not statistically different. The level of protection at the highest concentration virus is similar to challenge with Type 2 IHNV.

Type 5 IHNV challenge

A Cedar River isolate was chosen to test cross immunity to Type 5 IHNV strains. This isolate was used because of the virus is found in a region close to the Columbia River. Rainbow trout vaccinated with type 1 glycoprotein of IHNV were protected from the lethal effects of the Cedar River IHNV infection (Table 9, Figure 6). Differences were noted at all virus concentrations in the level of protection afforded by injection or immersion. These differences, however, are not statistically significant.

Again what is clearly demonstrated is the resulting protection of fish from heterologous IHN virus infection by the single strain vaccination.

DISCUSSION

The envelope glycoprotein of IHN virus has been purified and shown to induce protective immunity against challenge with either homologous or heterologous types of virulent IHN virus. Thus IHN virus is similar to rabies and vesicular stomatitis virus, both mammalian rhabdoviruses with envelope glycoproteins that are solely responsible for inducing neutralizing antibody and protective immunity in the host (Cox, Dietzschold, and Schneider, 1977).

The method of immunization does not lead to significant differences in protection in all but one experiment. Immersion immunization was almost as effective as inoculation at all levels of virus challenge. There appeared to be no difference in protection between immersion and inoculation delivery when fish were challenged with low concentrations of IHN virus. These low virus concentrations are representative of that found in the environment. The only case where immersion failed to be as effective as direct G protein injection was observed in the cross-challenge experiment with Type 2 IHN virus (Figure 3, Table 6). This result may be an artefact of the small number of fish in the experiment. A survey of the data does indicate that immersion is an effective and practical method of vaccination.

Vaccine-induced protection was more clearly demonstrated when the challenge virus dose was sufficient to produce an **LD₅₀** but not so great as to overwhelm the immune system. At dilutions of IHN virus of 1:10,000 and 1:100,000 the immunized fish were completely protected. At virus challenge dilutions of 1:100 to 1:1000 2- to 10-fold reduction in mortality still occurred in the vaccinated fish. Yet, control, unimmunized fish were killed at levels of 50%

to 100% (Fig. 1-6). Statistically significant protection was afforded by the G protein vaccination.

Type 1 IHNV is very virulent in Kokanee salmon and will produce 90% mortality at 1:100 dilution of the virus. In contrast, the same virus type in rainbow trout was less virulent and produced mortalities of 70% at 1:100 dilution and 50% at 1:1000 dilution. The vaccination of rainbow trout with purified glycoprotein was much more effective in inducing a protective response (Figures 1 and 2). It is possible that the virulence exhibited by Type 1 IHNV for kokanee salmon is an adaptation which permits that virus type to grow more rapidly in that species. In that case, it is probable that the virus would mount a pathogenic infection sufficiently rapid to overwhelm the fish immune response. Similar effects have been observed for foot-and-mouth disease vaccination trials (Kleid, personal communication).

Protection to the Type 2 IHNV (Hagerman Valley) infection demonstrated by a 2-3 fold-reduction in mortality. This level of protection is similar to the Type 1 Kokanee results (Figure 1 and 3). However with Type 3 IHNV (Elk River) a seven fold reduction in titer was achieved at a 1:100 dilution (Figure 4). This is analogous to the Type 1 Rainbow trout experiment.

Vaccination and subsequent challenge with Type 4 IHNV (Coleman River) gave results similar to Type 2 and Type 1 Kokanee experiments. A 2-fold level of protection was present at 1:100 dilution; nearly 7-fold reduction in mortality was found at 1:1000 dilution of IHNV.

The protection from heterologous challenge with Type 5 IHNV was somewhat intermediate to the protection described above, The reduction in mortality is 2 to 3 fold at all dilutions except the injected group of fish at 1:100,000 dilution where no mortalities occurred. This result may be caused by a virus strain that kills even at low dilutions. To protect against this greater virulence higher vaccine dosage or booster immunization may be required.

Protective immunity was induced by vaccination with 0.4-0.5 ng of purified IHNV glycoprotein. This quantity of viral protein is equivalent to an infectious dose of 2025 **TCID₅₀** units of virus. The estimate is based on a calculation of 22% of the total virus protein as glycoprotein (Leong, Hsu, and Engelking, 1983) and a molecular weight of 7.46×10^7 daltons for the virus particle. The protein dose:body weight ratio used in these experiments was 0.5 ug/0.5 g or $1/10^6$, a dose approximately equivalent to the dose normally used to immunize mice and rabbits with purified rabies glycoprotein (Cox et al., 1980 Wiktor et al., 1973). The protein dose used in the rabies studies ranged from 12.5 ug of purified protein per adult mouse (30-35 g body weight) to 50 pg per adult rabbit (1135 g body weight). These comparisons suggest that the IHNV glycoprotein is highly immunogenic in fish.

Although the duration of these experiments was carried only one month, the cumulative mortality data (Annual Report, 1984) suggest that the duration of immunity should last longer than that period of time. These studies did not include any experiments that accurately measure the duration of immunity because salmon and trout are refractory to the pathogenic effects of IHNV infection after 6 months (Pilcher and Fryer, 1980). A method to detect immunization by other means than virus challenge is being developed for these studies. ELISA and other immunological methods employing goat anti-trout globulin serum are being tested. In addition, the effect of a booster dose on the duration of immunity is being determined.

These experiments clearly demonstrate that the viral glycoprotein alone, devoid of any other component of the virus particle, is highly immunogenic. A second important point established by these experiments is that the IHNV variants (Type 1 to Type 5) have a conserved antigenic site(s) that allows a monovalent vaccine to elicit a protective response in fish to all IHNV

variants. Therefore, the production of a recombinant vaccine based on a single type of IHNV is possible. Fish in any geographic area may be protected from endemic as well as exotic IHNV types and the development of a more costly polyvalent vaccine may not be necessary.

Table 1. Description of IHNV variants used in cross-challenge protection studies.

Type	Location	Fish Species	Fish Tissue	Date of Isolation
1	Round Butte Hatchery Oregon	STS	Ovarian	1975
2	Hagerman Valley Idaho	RB	Adult	1978
3	Elk River Oregon	CHF	Fry	1979
4	Coleman Hatchery California	CH	Alevin	1980
5	Cedar River Washington	CHF	Ovarian	1981

Table 2. Molecular Weight Characteristics Used in Typing IHNV.

Protein	Type 1	Type 2	Type 3	Type 4.	Type 5
N	40.5	42.8	43.25	40.5-41.0	41.0-44.0
G	67.0	67.0	67.0	70.0	67 .0

The figures are molecular weight X 1000 daltons.

*Type 5 is less defined and N varies depending on the isolates.

LEGENDS

- Figure 1. Determination of the **LD₅₀** for control, nonvaccinated fish and fish vaccinated by injection or immersion with purified Glycoprotein of IHN Type 1. The fish were challenged by varying dilutions of virulent IHN Type 1 from Round Butte as described in Materials and Methods. The fish were Kokanee fry obtained from Wizard Falls Hatchery, Oregon.
- Figure 2. Determination of the **LD₅₀** for control, nonvaccinated fish and fish vaccinated by injection or immersion with purified Glycoprotein of IHN Type 1. The fish were challenged by varying dilutions of virulent IHN Type 1 from Round Butte as described in Materials and Methods. The fish were Rainbow trout fry obtained from Oak Springs Hatchery, Oregon.
- Figure 3. Determination of the **LD₅₀** for control, nonvaccinated fish and fish vaccinated by injection or immersion with purified Glycoprotein of IHN Type 1. The fish were challenged by varying dilutions of virulent IHN Type 2 from Hagerman Valley, Idaho as described in Materials and Methods. The fish were Rainbow trout fry obtained from Roaring River Hatchery, Oregon.
- Figure 4. Determination of the **LD₅₀** for control, nonvaccinated fish and fish vaccinated by injection or immersion with purified Glycoprotein of IHN Type 1. The fish were challenged by varying dilutions of virulent IHN Type 3 from Elk River, Oregon as described in Materials and Methods. The fish were Rainbow trout fry obtained from Roaring River Hatchery, Oregon.

Figure 5. Determination of the **LD₅₀** for control, nonvaccinated fish and fish vaccinated by injection or immersion with purified Glycoprotein of IHNV Type 1. The fish were challenged by varying dilutions of virulent IHNV Type 4 from Coleman River, California as described in Materials and Methods. The fish were Rainbow trout fry obtained from Roaring River Hatchery, Oregon.

Figure 6. Determination of the **LD₅₀** for control, nonvaccinated fish and fish vaccinated by injection or immersion with purified Glycoprotein of IHNV Type 1. The fish were challenged by varying dilutions of virulent IHNV Type 5 from Cedar River, Washington as described in Materials and Methods. The fish were Rainbow trout fry obtained from Roaring River Hatchery, Oregon.

Figure 7. SDS-polyacrylamide slab gel electrophoresis of the five types of IHNV. The first and last lanes, MWM, weight markers: phosphorylase B (92,500); bovine serum albumin (66,200); ovalbumin (45,000); carbonic anhydrase (31,000); soybean trypsin inhibitor (21,500); and lysozyme (14,400). Lane 2 from right purified IHNV from Round Butte Hatchery (Type 1): L (150,000); G (67,000); N (40,500); M-1 (25,000); and M-2 (22,500). Lane 3 purified IHNV from Hagerman Valley (Type 2); N (42,800). Lane 4 purified IHNV from Elk River (Type 3); N (43,250). Lane 5 purified IHNV from Coleman River (Type 4); G (70,000); N (40,500-41,000). Lane 6 purified IHNV from Cedar River (Type 5); N (43,000).

Table 3. Comparison of immersion and inoculation vaccination with IHN Type 1 glycoprotein in rainbow trout to challenge with IHN Round Butte Type 1.

Virus ^a Dilution	Inoculated				Immersed				Control		
	No	SL	%	RPS	No.	SL	%	RPS	No.	SL	%
-2	24	4	16	78	25	4	16	78	25	18	72
-3	25	3	12	77	25	4	16	69	25	13	52
-4	25	1	4	0	25	0	0	-	25	1	4
-5	25	0	0	-	25	0	0	-	25	0	0

No. = Number of fish in group.

SL = Specific loss, i.e. number of fish dying from IHN infection.

% = Percent mortality.

RPS = Relative percent survival (see Materials and Methods).

^aVirus dilutions are shown as ten-fold dilutions of a stock of virus (ca. 2×10^8 TCID₅₀/ml).

Table 4. Comparison of immersion and inoculation vaccination with IHN Type 1 glycoprotein in salmon to challenge with IHN Round Butte Type 1.

Virus ^a Dilution	Inoculated				Immersed				Control		
	No	SL	%	RPS	No.	SL	%	RPS	No.	SL	%
-2	100	36	36	58	100	51	51	41	100	86	86
-3	100	18	18	77	100	27	27	65	97	75	77
-4	99	5	5	85	100	7	7	79	100	34	34
-5	100	4	4	67	96	3	3	75	100	12	12

Combined results of four experiments

No. = Number of fish in group.

SL = Specific loss, i.e. number of fish dying from IHN infection.

% = Percent mortality.

RPS = Relative percent survival (see Materials and Methods).

^aVirus dilutions are shown as ten-fold dilutions of a stock of virus (ca. 2×10^8 TCID₅₀/ml).

Table 5. A comparison of immersion and inoculation vaccination for IHN Type 1 glycoprotein. Cross Protection to Type 2, Hagerman Valley, Idaho.

Kokanee fry were vaccinated with Type 1 glycoprotein and challenged with Type 5 IHN at dilutions indicated.

Virus ^a Dilution	Inoculated				Immersed				Control		
	No	SL	%	RPS	No.	SL	%	RPS	No.	SL	%
-2	24	10	42	51	25	8	32	62	20	17	85
-3	12	8	62	13	23	9	39	45	17	12	71
-4	9	1	11	82	5	3	60	0	25	15	60
-5	20	5	25	50	15	6	40	20	24	12	50

No. = Number of fish in group.

SL = Specific loss, i.e. number of fish dying from IHN infection.

% = Percent mortality.

RPS = Relative percent survival (see Materials and Methods).

^aVirus dilutions are shown as ten-fold dilutions of a stock of virus (ca. 2×10^8 TCID₅₀/ml).

Table 6. A comparison of immersion and inoculation vaccination for IHN Type 1 glycoprotein. Cross Protection to Type 2, Hagerman Valley, Idaho.

Rainbow Trout fry were vaccinated with Type 1 glycoprotein and challenged with Type 2 IHN at dilutions indicated.

Virus ^a Dilution	Inoculated				Immersed				Control		
	No	SL	%	RPS	No.	SL	%	RPS	No.	SL	%
-2	9	6	67	67	11	10	91	91	12	12	100
-3	10	4	40	43	11	7	64	70	12	11	92
-4	9	2	22	29	10	4	40	53	12	9	75

No. = Number of fish in group.

SL = Specific loss, i.e. number of fish dying from IHN infection.

% = Percent mortality.

RPS = Relative percent survival (see Materials and Methods).

^aVirus dilutions are shown as ten-fold dilutions of a stock of virus (ca. 2×10^8 TCID₅₀/ml).

Table 7. A comparison of immersion and inoculation vaccination for IHN Type 1 glycoprotein. Cross Protection to Type 3, Elk River, Oregon.

Rainbow Trout fry were vaccinated with Type 1 glycoprotein and challenged with Type 3 IHN at dilutions indicated.

Virus ^a Dilution	Inoculated				Immersed				Control		
	No	SL	%	RPS	No.	SL	%	RPS	No.	SL	%
-2	25	4	16	75	25	1	4	94	25	16	64
-3	25	0	0	-	25	3	12	80	25	15	60
-4	25	0	0	-	25	0	0	-	25	8	32
-5	25	0	0	-	25	0	0	-	25	1	4

No. = Number of fish in group.

SL = Specific loss, i.e. number of fish dying from IHN infection.

% = Percent mortality.

RPS = Relative percent survival (see Materials and Methods).

^aVirus dilutions are shown as ten-fold dilutions of a stock of virus (ca. 2×10^8 TCID₅₀/ml).

Table 8. A comparison of immersion and inoculation vaccination for IHN Type 1 glycoprotein. Cross Protection to Type 4, Coleman River, California.

Rainbow Trout fry were vaccinated with Type 1 glycoprotein and challenged with Type 4 IHN at dilutions indicated.

Virus ^a Dilution	Inoculated				Immersed				Control		
	No	SL	%	RPS	No.	SL	%	RPS	No.	SL	%
-2	25	8	32	57	25	8	32	57	20	15	75
-3	25	4	16	80	25	5	20	75	20	16	80
-4	25	2	8	84	25	2	8	84	20	10	50
-5	25	0	0	-	25	0	0	-	20	5	25

No. = Number of fish in group.

SL = Specific loss, i.e. number of fish dying from IHN infection.

% = Percent mortality.

RPS = Relative percent survival (see Materials and Methods).

^aVirus dilutions are shown as ten-fold dilutions of a stock of virus (ca. 2×10^8 TCID₅₀/ml).

Table 9. A comparison of immersion and inoculation vaccination for IHN Type 1 glycoprotein. Cross Protection to Type 5, Cedar River, Washington.

Rainbow Trout fry were vaccinated with Type 1 glycoprotein and challenged with Type 5 IHN at dilutions indicated.

Virus ^a Dilution	Inoculated				Immersed				Control		
	No	SL	%	RPS	No.	SL	%	RPS	No.	SL	%
-2	22	9	41	59	25	10	40	56	25	25	100
-3	22	7	32	67	25	9	36	63	25	24	96
-4	21	4	19	75	25	7	28	63	25	19	76
-5	22	0	0	-	25	3	12	70	25	10	40

No. = Number of fish in group.

SL = Specific loss, i.e. number of fish dying from IHN infection.

% = Percent mortality.

RPS = Relative percent survival (see Materials and Methods).

^aVirus dilutions are shown as ten-fold dilutions of a stock of virus (ca. 2×10^8 TCID₅₀/ml).

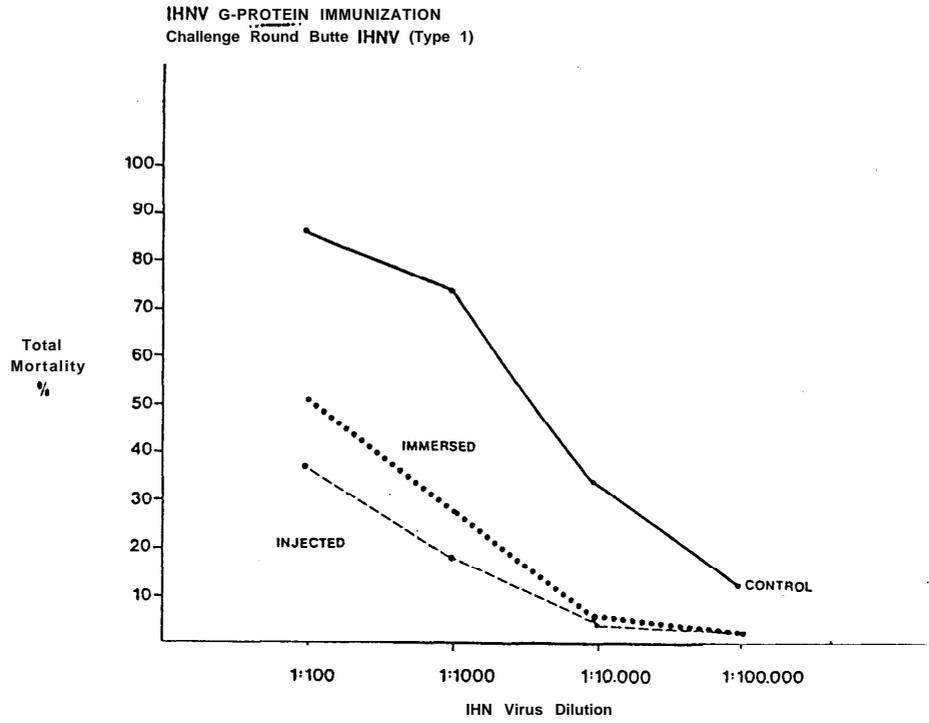


Figure 1.

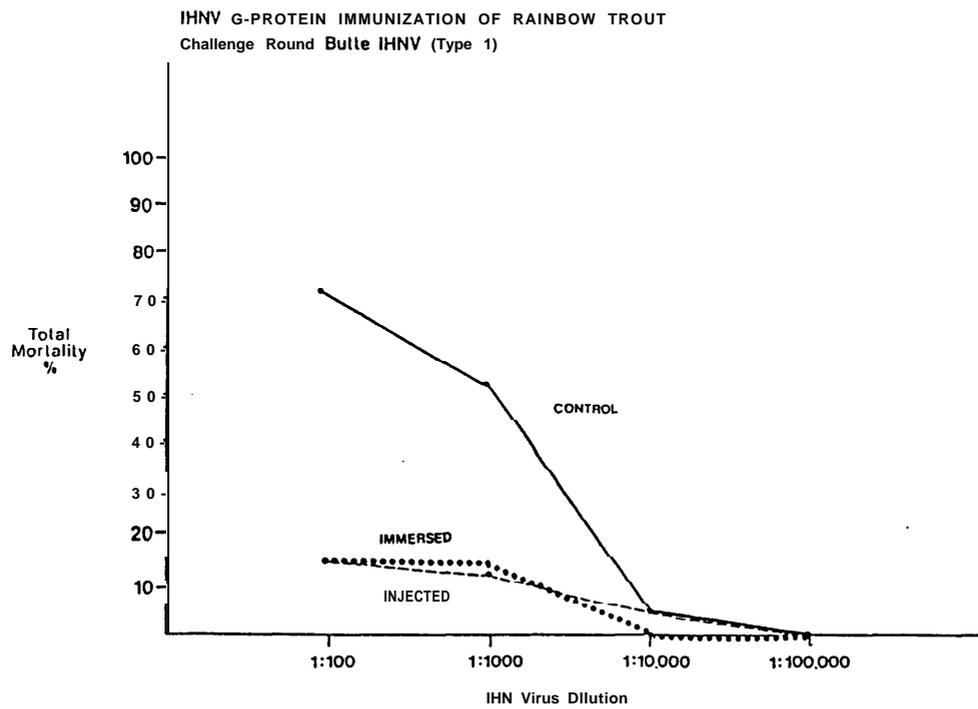


Figure 2.

IHNV G-PROTEIN IMMUNIZATION
 Cross Challenge Hagerman Valley IHNV (Type 2)

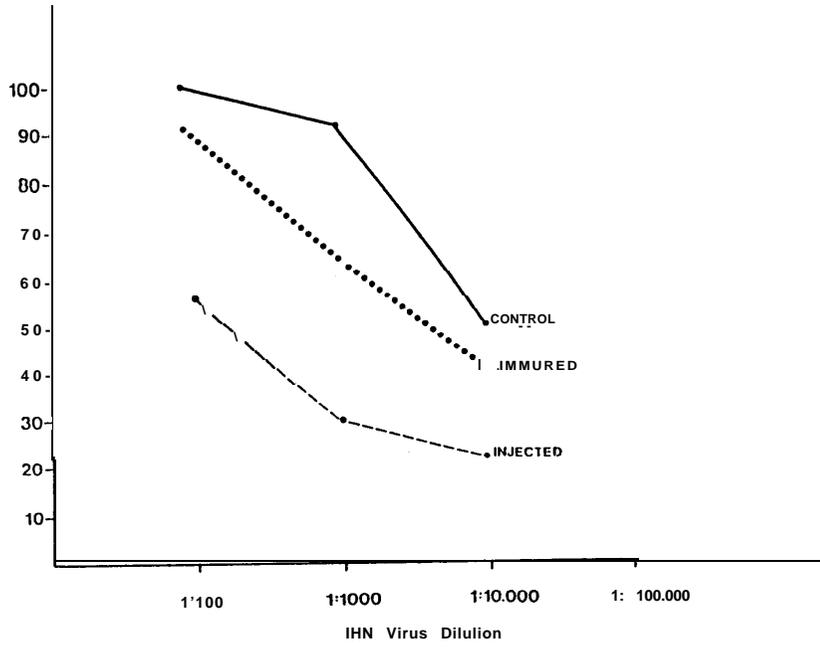


Figure 3.

IHNV G-PROTEIN IMMUNIZATION
 Cross Challenge Elk River IHNV (Type 3)

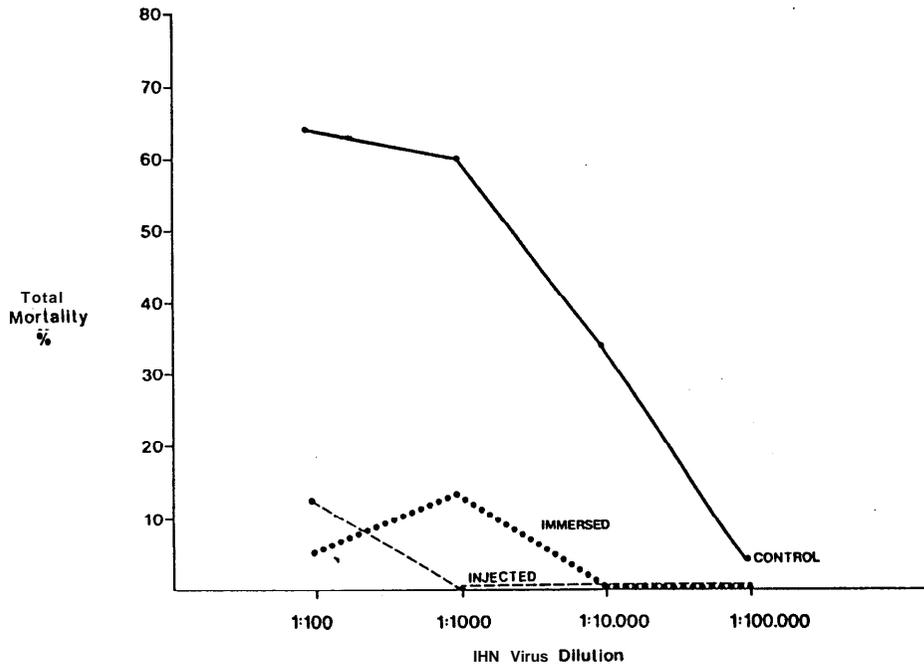


Figure 4.

IHNV G-PROTEIN IMMUNIZATION
 Cross Challenge Coleman River IHNV (Type 4)

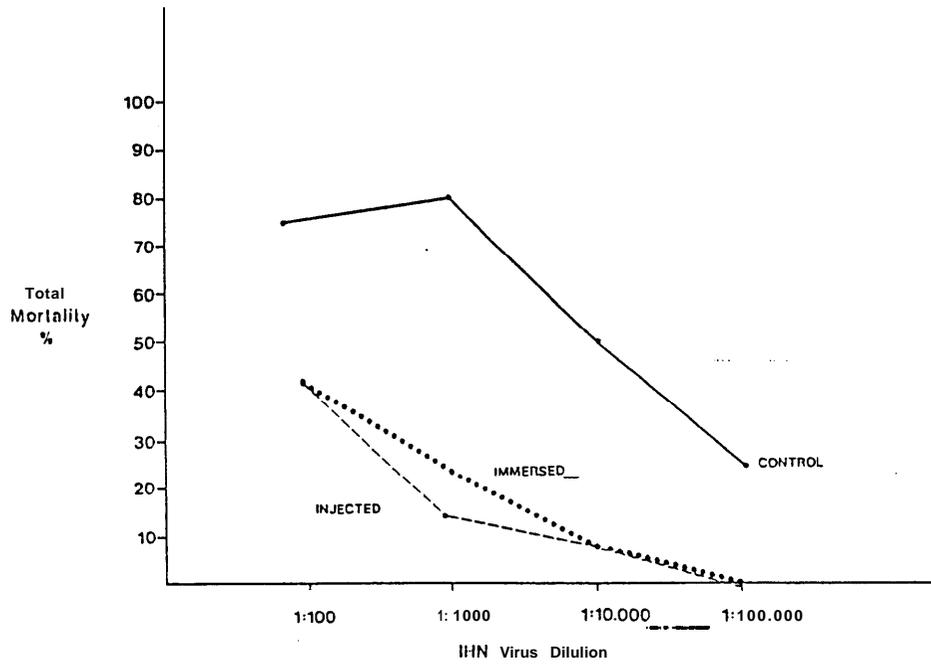


Figure 5.

IHNV G-PROTEIN IMMUNIZATION
 Cross Challenge Cedar River IHNV (Type 5)

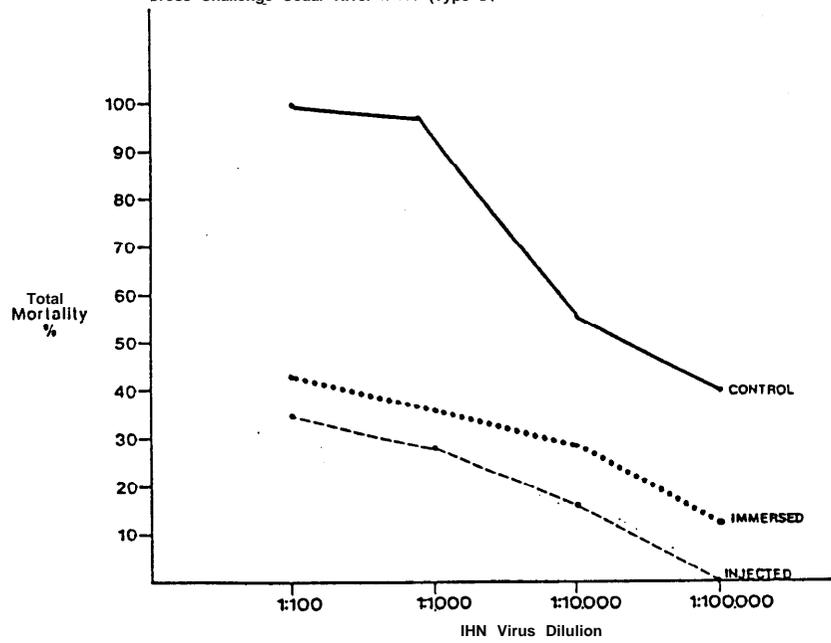


Figure 6.

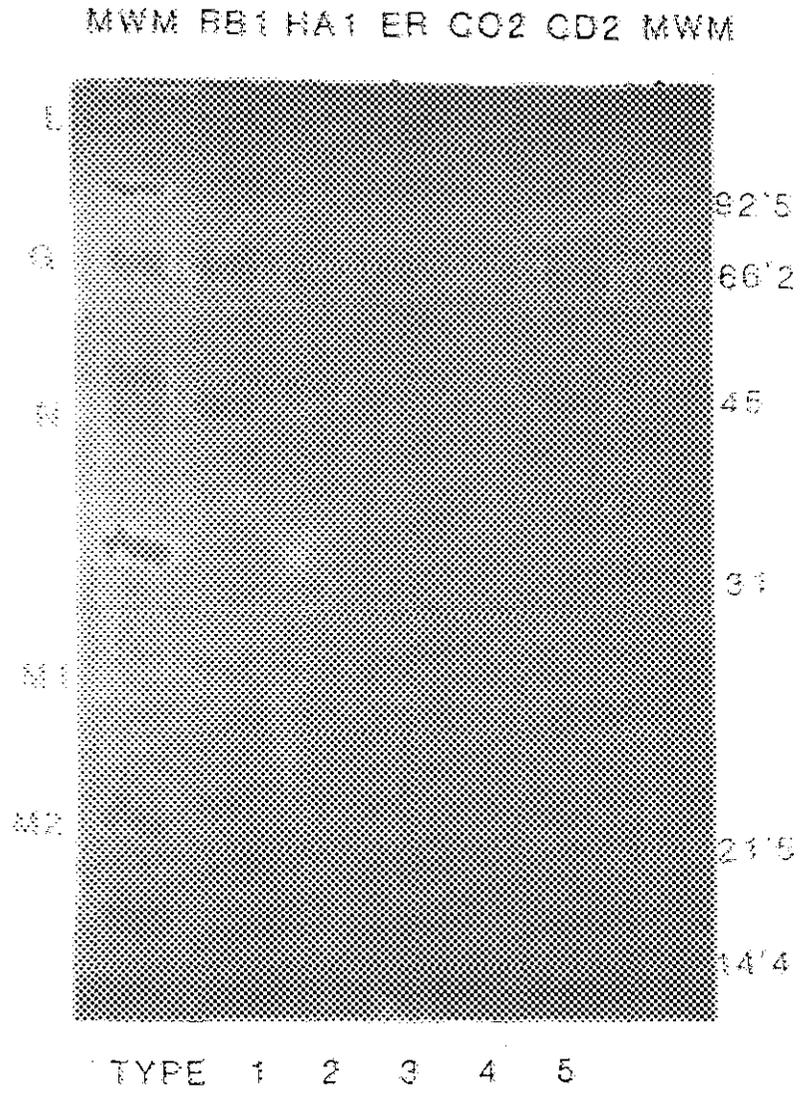


Figure 7.

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