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To cite this version:
Se Lapatra, Ka Lauda, Gr Jones. Aquareovirus interference mediated resistance to infectious hematopoietic necrosis virus. Veterinary Research, BioMed Central, 1995, 26 (5-6), pp.455-459. hal-00902374

HAL Id: hal-00902374
https://hal.archives-ouvertes.fr/hal-00902374
Submitted on 1 Jan 1995

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Aquareovirus interference mediated resistance to infectious hematopoietic necrosis virus

SE LaPatra *, KA Lauda, GR Jones

Clear Springs Foods Inc, PO Box 712, Buhl, ID 83316 USA

Summary — This study investigated the stimulation of specific host defenses to IHNV that are created by prior exposure to an avirulent reovirus (chum salmon reovirus; CSV). Preexposure of rainbow trout to CSV for 1 h resulted in a relative percent survival that ranged from 68 to 100% when fish were challenged with IHNV over an 8 week period. The levels of serum neutralizing antibodies detected 28 d after the IHNV challenge were significantly lower among fish receiving prior exposures to CSV than among non-CSV-treated fish. The differences in the humoral response to IHNV in the CSV-treated fish suggested that other immune defense mechanisms may be involved.

INTRODUCTION

Dual infection of rainbow trout Oncorhynchus mykiss with 2 viruses has previously been reported but the advantageous effects elicited by these agents on the host's immune system have not been explained. De Kinkelin et al (1992) were able to show that rainbow trout that have been preexposed to infectious pancreatic necrosis virus (IPNV) and later challenged with viral hemorrhagic septicemia virus (VHSV) showed significant resistance to VHSV compared with fish not previously exposed to IPNV. The basis for this virus interference mediated resistance was sus-
pected to be interferon, although this could not be definitively demonstrated. More recently, it has been shown that preexposure of rainbow trout to the cutthroat trout virus (CTV) decreased their susceptibility to subsequent challenges with infectious hematopoietic necrosis virus (IHNV). This heterologous antiviral protection was observed up to 4 weeks following the CTV exposure. In addition, the concentrations of serum anti-IHNV neutralizing antibodies were significantly higher among fish previously exposed to CTV when compared with mock-treated groups that were challenged with IHNV. Although the exact mechanism of protection induced by CTV is unknown, the virus was also shown to be an inducer of interferon-like activity in rainbow trout anterior kidney cells (Hedricks et al, 1994).

This study was conducted to evaluate the chum salmon reovirus (CSV) (Winton et al, 1981) and its capacity to stimulate host defenses against a viral infection. Reoviruses have been shown to be potent antiviral cytokine inducers in other systems and have been detected in aquatic animals worldwide.

**MATERIALS AND METHODS**

**Cell cultures**

Two fish cell lines were used for the isolation, propagation, quantitation and identification of IHNV and CSV isolates used in this study: (1) the CHSE-214 line (American Type Culture Collection; ATCC CRL 1681) from chinook salmon embryos (Lannan et al, 1984); and (2) Epithelioma papulosum cyprini cells (EPC) from common carp Cyprinus carpio (Fijan et al, 1983). The propagation methods have been described previously (Winton et al, 1981; LaPatra et al, 1989). The viruses used for the virus interference mediated resistance trials were grown in CHSE-214 cells at 18°C in MEM-2 (2% fetal bovine serum) and stored at −75°C.

**Viruses**

The chum salmon reovirus was originally isolated in 1978 from apparently healthy adult chum salmon O keta in Hokkaido, Japan by Winton et al (1981). The 220-90 strain of infectious hematopoietic necrosis virus (IHNV) was isolated from juvenile rainbow trout in the Hagerman Valley, ID, USA (LaPatra et al, 1991).

**Fish challenges**

Rainbow trout were obtained from Clear Springs Foods Inc, Broodstock Operations. The first evaluation consisted of exposing 1 group of rainbow trout (mean weight, 8.8 g) to 10⁴ TCID₅₀ (50% tissue culture infective dose)/ml CSV for 1 h and mock exposure of a duplicate group. These fish were put on identical feeding regimes and held for 1 week. Groups of fish from both treatments were challenged with IHNV using standard procedures (LaPatra et al, 1991). Subgroups from each treatment were mock exposed to the virus and served as negative controls. All groups were monitored for 5 weeks and a portion of the dead fish were examined for the virus.

To test the duration of the protection against IHNV after the preexposure of the fish to CSV, groups of 500 fish (mean weight, 5.2 g) were exposed to 10⁴ TCID₅₀/ml CSV for 1 h or mock-exposed. Duplicate groups of 20 or 25 fish from each treatment were challenged with 10⁴–10⁵ pfu (plaque-forming units)/ml IHNV at weeks 1, 2, 4, 6 and 8. The relative percent survival (RPS) for the CSV exposed and then IHNV challenged groups was calculated by the following formula:

\[ RPS = 1 - \frac{\text{cumulative % mortality CSV exposed}}{\text{cumulative % mortality mock-treated}} \times 100 \]

**Complement dependent neutralization tests**

Sixty survivors from each treatment and 20 mock-infected controls from the first challenge trial were bled in 2-fish pools and the serum was titered for IHNV neutralization activity. Similar plaque reduction procedures for IHNV have been reported.
(Hattenberger-Baudouy et al., 1989; Jorgensen et al., 1991; LaPatra et al., 1993). Briefly, the rainbow trout serum was combined with a suspension of IHNV. The specific antibodies that were present bound to the virus. Fish complement was added to complete the antigen–antibody reaction which caused the virus to be neutralized. The reduction in the amount of infectious virus was quantitated by EPC plaque assay procedures and neutralization titers were calculated for each serum sample tested. Serum-neutralization titers obtained from the fish in each treatment were compared by the Chi-square test.

Isolation and quantitation of virus

We examined a minimum of 20% of each day’s mortality for the virus (LaPatra et al., 1991). The quantitation of the virus used for the fish exposures or isolated from dead fish was accomplished by plaque assay procedures (LaPatra et al., 1989). The virus concentration in the kidney-spleen-liver homogenate was determined for a portion of the dead fish examined in each test.

<table>
<thead>
<tr>
<th>Week</th>
<th>Cumulative percent mortality</th>
<th>Relative survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CSV exposed (%)</td>
<td>Mock exposed (%)</td>
</tr>
<tr>
<td>1</td>
<td>4 (2/50)</td>
<td>42 (21/50)</td>
</tr>
<tr>
<td>2</td>
<td>10 (5/49)</td>
<td>92 (45/49)</td>
</tr>
<tr>
<td>4</td>
<td>0 (0/50)</td>
<td>86 (43/50)</td>
</tr>
<tr>
<td>6</td>
<td>6 (3/50)</td>
<td>45 (23/51)</td>
</tr>
<tr>
<td>8</td>
<td>13 (5/40)</td>
<td>40 (16/40)</td>
</tr>
</tbody>
</table>

| Fish were treated with either culture media from uninfected cells (mock) or CSV for 1 h. At 1, 2, 4, 6 and 8 week post-treatment, 2 groups of 20 or 25 fish from each treatment group were challenged with IHNV strain 220-90 and monitored 21 d for mortality. * Fraction of fish that died. |

RESULTS AND DISCUSSION

Cumulative mortality detected in the CSV exposed and IHNV challenged group was 9% (26/300) and 26% (79/300) in the IHNV challenged only group. The relative percent survival was 65%, which is consistent with results obtained in a previous experiment that investigated interference mediated IHNV resistance with a picorna-like virus, CTV (Hedrick et al., 1994). The protection afforded by 1 h bath exposures to CTV provided up to 69% RPS following IHNV challenge. This effect was present at 1, 2 and 4 weeks post-exposure to CTV but was absent when the fish were tested at 6 weeks (Hedrick et al., 1994). Major differences were observed in the cumulative percent mortality between our 2 treatment groups at each of the time points tested (table I). Strong protection was still observed in fish that were preexposed to CSV 8 weeks post-exposure. Infectious hematopoietic necrosis virus was reisolated from 83% (89/107) of the dead fish examined with a mean titer 10^6.6 pfu/g.

No IHNV neutralizing activity was detected in any of the control fish. However, the CSV-exposed IHN survivors had significantly (P < 0.001) lower neutralization titers than the surviving fish exposed only to IHNV (table II). This is contrary to what was observed during the course of similar experiments with the CTV. The levels of IHNV antibodies were significantly higher among the fish receiving prior exposure to CTV than among the non-CTV-treated fish challenged with IHNV (Hedrick et al., 1994).

These results indicated that not only was CSV capable of providing excellent protection to IHNV challenge but specific immunity (serum neutralizing activity) also developed. However, the response appeared to be significantly depressed in treated fish.

Several possibilities for the CSV stimulation of nonspecific immune functions have been postulated. These include interferon induction and/or stimulation of the
macrophage or natural killer cell functions. Cytokine activity is central to these responses but unfortunately, in salmonids, many of these functions are poorly understood. As has been demonstrated for CTV (Hedrick et al., 1994), CSV has been shown to be a potent inducer of anti-viral-like activity in the anterior kidney cells isolated from rainbow trout (JL Congelton, National Biological Survey, University of Idaho, Moscow, ID, USA, personal communication). It is possible that cell-mediated immunity was also induced by CSV and provided protection against IHNV. A preliminary model, which was postulated based on our initial results, suggested that CSV induced the release of a T-cell cytokine that enhanced the cell-mediated immunity through the stimulation of \( T_{cytotoxic} \) cells. This was supported by our IHNV neutralization titer data.

A low level of mortality has also been observed in fish stocks exposed to CSV (data not shown). Gross signs of a potential viral infection were observed and CSV was isolated from dead fish where high concentrations (\( 10^6 \) to \( 10^7 \) pfu/ml) of the virus were detected. Randomly collected histological specimens were analyzed. In previous studies that examined the pathogenesis of CSV, no death occurred in 1–2 g chum, chinook, or kokanee salmon fry or rainbow trout injected with \( 10^4 \) TCID\(_{50}\)/ml. However, a slight focal necrotizing hepatitis was observed in the liver sections taken from infected fish (Winton et al., 1989). Multi-focal to complete liver necrosis was observed in our specimens (RP Hedrick, University of California, Davis, USA, personal communication). Microscopic tissue changes were also observed in the endothelial cells and sinusoids of the kidney. We have also been able to detect CSV in fish 42 d post-exposure as previously reported (Winton et al., 1989). The chum salmon reovirus appears to be very successful at establishing a persistent infection that is possibly confined to the liver. A non-lymphoid chronic acute-phase response may account for the RPS results for fish previously exposed to CSV (Bayne, 1994). By examining the levels of non-lymphoid (‘natural’) immune defense factors in the sera from CSV and mock-exposed fish it might be possible to obtain information supporting this hypothesis.

Table II. Susceptibility of rainbow trout \( O\) mykiss to infectious hematopoietic necrosis virus (IHNV) following exposure to the chum salmon reovirus (CSV) and resulting serum neutralization titer.

<table>
<thead>
<tr>
<th>CSV preexposed (%)</th>
<th>Mock preexposed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IHNV challenged</td>
</tr>
<tr>
<td>CPM(^a)</td>
<td>9</td>
</tr>
<tr>
<td>Mean titer</td>
<td>63(^*)</td>
</tr>
<tr>
<td>RPS(^b)</td>
<td>65</td>
</tr>
</tbody>
</table>

Fish were challenged with CSV strain 220–90 at a dose of approximately \( 10^4 \) pfu/ml. Mean serum antibody titers were determined for 30 two-fish pools from both IHNV-exposed groups and 10 two-fish pools from each of the other control groups at 4 weeks post-challenge with IHNV. Titers are expressed as the reciprocal of the serum dilution that resulted in a 50% reduction in the average number of plaques detected in the negative control. The antibody titers of CSV pre-exposed fish were significantly lower (\( * P < 0.001 \)) than the mock preexposed group following IHNV challenge. \(^a\) CPM = cumulative percent mortality; \(^b\) RPS = relative percent survival when compared to the mock-treated group.
Our results have shown that CSV provides protection for up to 8 weeks. We are now undertaking further studies that will attempt to define the mechanism by which this protection may occur. Better understanding of this mechanism may result in the development of more effective viral control strategies such as vaccines. In addition, it would generate more information about salmonid immunology.

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