Channel catfish reovirus (CRV) inhibits replication of channel catfish herpesvirus (CCV) by two distinct mechanisms: viral interference and induction of an anti-viral factor

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ABSTRACT: Catfish reovirus (CRV), a double-stranded RNA virus, inhibited channel catfish herpesvirus (CCV) replication by 2 different mechanisms: (1) directly as a consequence of its own replication, and (2) indirectly due to the induction of an anti-viral factor. In the former, prior infection with CRV significantly reduced subsequent CCV protein synthesis and virus yield. CRV-mediated interference was greatest when CRV infection preceded CCV infection by 16 h, and was least when cell cultures were simultaneously infected with both viruses. In the latter case, infection of channel catfish ovary (CCO) cultures with UV-inactivated CRV resulted in the synthesis (or release) of an anti-viral factor. Cells producing the factor were protected from CCV infection, as were cells which had been treated with spent culture medium containing anti-viral activity. Interestingly an anti-viral activity was constitutively present in long-term cultures of catfish T cells and macrophages. Whether this factor and the one induced by UV-inactivated CRV are identical is not known, but analogy to mammalian systems suggests that the former may be similar to type II interferon, whereas the latter may be the piscine equivalent of type I interferon. These results suggest that UV-inactivated CRV may prove useful in the induction and characterization of interferon-like anti-viral proteins in the channel catfish and that long-term cultures of catfish T cells and monocytes may serve as a ready source of additional anti-viral factors.

KEY WORDS: Viral interference · Anti-viral factor · Interferon · Catfish reovirus · Channel catfish herpesvirus · Catfish lymphoid cell lines

INTRODUCTION

Viruses are capable of inhibiting not only cellular macromolecular synthesis, but also the replication of co-infecting heterologous or homologous viruses. The ability of one virus to interfere with the replication of another virus is termed viral interference and is due to one of several mechanisms (Younger & Whitaker-Dowling 1994). (1) Infection with one virus may inhibit replication of a second virus by blocking virus entry due to down-regulation of viral receptors or to competition between viruses for a common receptor (Steck & Rubin 1966). (2) Alternatively, replication by the initially infecting virus may inhibit host cell functions that

are required by the super-infecting virus or the first virus may block heterologous virus replication directly. For example, poliovirus blocks replication of super-infecting vesicular stomatitis virus (VSV) by inhibiting VSV translation, whereas VSV inhibits replication of super-infecting influenza A virus by blocking influenza virus transcription (Younger & Whitaker-Dowling 1994). Likewise DNA viruses also block replication of super-infecting viruses. The iridovirus frog virus 3 (FV3) inhibits replication of vaccinia virus, VSV, and equine herpesvirus type 1 (Aubertin et al. 1970, Tannenbaum et al. 1979, Chinchar & Caughman 1986). (3) Finally, interference may also be mediated by defective interfering particles or temperature sensitive (*ts*) mutants that inhibit the replication of wild type virus (Ramig 1990).

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In addition to the above mechanisms, viruses also block replication of a second virus by induction of interferon or other anti-viral factors. Although interferon-mediated anti-viral effects are well characterized in mammals (Weissman & Weber 1986, Marcus 1994, Vilcek & Sen 1996), much less is known about the role they play in lower vertebrates (Yano 1996). Interferon-like activities have been identified in a variety of fish species, but definitive identification based on physiochemical and genetic properties is lacking (Beasley et al. 1966, Oie & Loh 1971, De Sena & Rio 1975, Eaton 1990, Graham & Secombes 1990, Rogel-Gaillard et al. 1993, Snegaroff 1993, Yano 1996). Furthermore, despite considerable effort, interferon genes from lower vertebrates (i.e. fish, amphibians, and reptiles) have not yet been cloned and sequenced.

Because reoviruses are good inducers of interferon (Oie & Loh 1971, Sekellick et al. 1994, Nibert et al. 1996), we examined the ability of channel catfish reovirus (CRV; Family *reoviridae*; genus *aquareovirus*) to block replication of channel catfish herpesvirus (CCV) following either productive infection or exposure to UV-inactivated CRV (UV-CRV). We observed that productive infection with CRV blocked subsequent replication of CCV and that exposure to UV-CRV induced the synthesis (or release) of an anti-viral factor that inhibited CCV replication. Moreover, several long-term catfish lymphoid cell lines constitutively synthesized a functionally similar anti-viral factor. These results extend the phenomenon of viral interference to viruses infecting catfish. Furthermore, they suggest that UV-CRV may be an excellent inducer of anti-viral proteins in teleosts and that catfish T cell and macrophage lines may be a good source of anti-viral cytokines.

METHODS

Cells and viruses. Channel catfish ovary (CCO) and brown bullhead (BB) cells were obtained from L. Hanson (College of Veterinary Medicine, Mississippi State University, USA) and the American Type Culture Collection (CCL 59), respectively. Both cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal calf serum (FCS). Catfish long-term lymphoid cell lines 42TA, CF-59, and 1G8 were established and maintained in AL medium supplemented with 5% catfish serum (AL5) as described by Miller et al. (1994a). 42TA is a macrophage cell line (Clem et al. 1996), 1G8 is a clonal B cell line (Miller et al. 1994b, Wilson et al. 1997), and CF-59 is a putative T cell line that arose after stimulation with phorbol 12-myristate-13-acetate/calcium ionophore. Cell lines were cultured at 27°C in a humidified incubator in the presence of 5% CO₂.

CCV was obtained from the American Type Culture Collection (Auburn 1, clone A strain, VR-665) and propagated in CCO cells at 28°C (Bowser & Plumb 1980a, b). Viral titers were determined by plaque assay on CCO cells. CRV (Amend et al. 1984), obtained from R. Hedrick (University of California, Davis, USA), was grown either in BB or CCO cells and the virus titer, i.e. the log₁₀ 50% tissue culture infectious dose (TCID₅₀), calculated by the method of Reed & Muench (1938). Because in our hands CRV does not plaque on CCO cells, the CCV titer in mixed infections of CCV and CRV was determined by plaque assay on CCO cells.

Viral interference assays. CCO cells were infected with CRV or UV-CRV at a nominal multiplicity of infection (MOI) of 0.1 and 1.0 TCID₅₀ cell⁻¹ either 16 h before or at the same time as infection with CCV. Virus was allowed to adsorb for 1 h, after which time the inoculum was removed and the cultures re-fed with growth medium. For radiolabeling viral proteins, growth medium lacking unlabeled methionine but containing [³⁵S]methionine (~25 Ci ml⁻¹) was added 4 h after CCV infection (i.e. 20 h after CRV infection). After 2 h the labeling medium was removed, and the cells solubilized in buffer containing sodium dodecyl sulphate (SDS) and 2-mercaptoethanol (Laemmli 1970). Radiolabeled proteins were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and visualized by autoradiography (Laemmli 1970). The development of cytopathic effects (CPE) was monitored in single and mixed infections by microscopy, and CCV replication was quantitated by plaque assay on CCO cells.

Induction of an anti-viral factor by UV-CRV. CRV (3 ml per 100 mm tissue culture dish) was inactivated by UV irradiation (150 mJ) using a Bio-Rad GS Gene-Linker. Eight day old CCO cultures were infected with UV-CRV at a nominal MOI of 0.1 and 1.0 TCID₅₀ cell⁻¹. Virus was allowed to attach for 1 h, after which the inoculum was removed and the cultures incubated at 27°C in DMEM containing 2 to 5% FCS. At the indicated times post infection, the culture medium was harvested and assayed for anti-viral activity in a challenge assay (see below). Where noted, mock- and UV-CRV-treated CCO cultures were infected with CCV and monitored for CCV-induced CPE and virion production 48 h later.

Challenge assays. A challenge assay, similar to that described by Renault et al. (1991), was used to determine whether anti-viral activity was present in spent culture medium (SCM) harvested from UV-CRV-infected CCO cells or catfish lymphoid cell lines. Approximately 10⁵ CCO cells in 100 µl of growth medium were seeded into 96-well plates containing an equal volume of increasing 2-fold dilutions of SCM. After overnight incubation, the cultures were challenged by

addition of 50 μ l of medium containing ~1800 plaque-forming units (PFU) CCV to each well and incubated at 28°C for an additional 48 h. At that time the medium was removed and the cultures stained with 1% crystal violet in 70% ethanol. After 5 min, the dye was removed and the plates washed with water until no residual dye remained. Anti-viral titers were calculated by multiplying the last dilution that showed >50% protection of the monolayer by 2 (to reflect the addition of an equal volume of cell suspension) and expressed as inhibitory units (IU) per milliliter.

RESULTS

Viral interference: CRV protects CCO cells from rapid lysis by CCV

To determine the role that innate immunity plays in protecting teleosts from virus infection, we examined the possibility that interferon, or an interferon-like molecule, protected catfish cells against CCV infection. The ability of SCM from CRV-infected CCO cultures to block CCV replication was monitored by challenge assay as described in 'Methods'. We observed that CCO cells exposed to SCM from CRV-infected CCO cultures were protected against challenge with CCV (Fig. 1, rows 7 and 8, and row 14). In contrast, monolayers receiving SCM from mock-infected cells (data not shown) or medium alone (Fig. 1, row 12) were susceptible to infection. The protective effect of the SCM was stable at pH 2 but was lost after heating (30 min at 56°C) or following high speed centrifugation (130 000 $\times g$, 2 h). These results, together with the observation that low dilutions of SCM from CRV-infected cultures induced CPE (Fig. 1, rows 3, 4, and 13), suggested that the anti-viral component was particulate and most likely infectious CRV rather than a soluble molecule such as interferon.

To confirm this result and to determine at what level CRV blocked CCV replication, CCO cells were exposed to either infectious CRV, UV-CRV, or a post-virion fraction obtained after centrifugation at 200 000 $\times g$ (S200), and challenged with CCV 16 h later. As controls, CRV infection alone, CCV infection alone, or

simultaneous CRV and CCV infections were performed. To determine the impact of CRV infection on subsequent CCV replication, 3 assays were employed: (1) viral protein synthesis was monitored from 4 to 6 h post CCV infection (i.e. 20 to 22 h post CRV infection) by radiolabeling infected cultures with [³⁵S]methionine and analyzing radiolabeled proteins by SDS-PAGE; (2) syncytium formation, characteristic of a productive CCV infection, was monitored microscopically by examination of infected cells 24 h after CCV infection; and (3) CCV replication was quantitated by plaque

Fig. 1. Infectious CRV protects CCO cells from CCV challenge. Spent culture medium from CRV-infected CCO cells (CRV) was monitored in a challenge assay along with medium from CRV-infected cells that had been heat-inactivated (Δ CRV), exposed to pH 2 for 24 h (pH2/CRV), or centrifuged at 130 000 $\times g$ for 2 h (S130/CRV). As a control, DMEM containing 2 or 5% FCS (D2 or D5) was also tested. The indicated media were serially diluted 2-fold (i.e. wells on the extreme left contain a 1:2 dilution, whereas those on the extreme right represent a dilution of 1:4096) and an equal volume (i.e. 100 μ l) of CCO cell suspension added. After overnight incubation, 50 μ l of medium containing ~1800 pfu CCV was added to the indicated wells. After 48 h, the medium was removed and the cells stained with crystal violet. Protection is indicated by an intact, dye-stained monolayer (e.g. rows 1 and 2), whereas CPE and destruction of the monolayer is indicated by the absence of dye (e.g. rows 12 and 18). In the top 2 panels, duplicate samples were uninfected (rows 1 to 6) or challenged with CCV (rows 7 to 10). In the bottom panel, duplicate samples were mock-infected (-) or challenged with CCV (+)

assay. We observed that prior infection with infectious CRV markedly reduced CCV protein synthesis in doubly infected cells (Fig. 2). Reduction of CCV protein synthesis was dependent upon the MOI of both CRV and CCV; higher inputs of CRV resulted in correspondingly higher levels of CCV inhibition, whereas elevated levels of CCV gave rise to more resistance to CRV-induced inhibition. We did not observe the synthesis of CRV-specific proteins in cells infected with UV-CRV or the S200 fraction, indicating that UV-irradiation and high-speed centrifugation resulted, respectively, in the inactivation and removal of CRV virions. However, despite the absence of CRV protein synthesis, a slight inhibition in the level of early and late CCV proteins was noted. Furthermore, in cultures simultaneously infected with both CRV and CCV, CCV

Fig. 2. Prior CRV infection blocks CCV protein synthesis. CCO cells were infected with $0.3 \text{ TCID}_{50} \text{ cell}^{-1}$ of CRV and subsequently super-infected with $0.8 \text{ pfu cell}^{-1}$ CCV immediately (lane 7) or 16 h later (lane 6). As controls, CCO cells were mock-infected (lane 5), or infected with CRV (lane 1) or CCV alone (lane 4). In addition, cells were infected with UV-CRV (lane 2) or an S200 fraction prepared from infectious CRV (lane 3) at a nominal MOI of $0.3 \text{ pfu cell}^{-1}$. Where indicated, cultures were super-infected with CCV 16 h after infection with UV-CRV or S200/CRV (lanes 8 and 9 respectively). Cells were radiolabeled with $[^{35}\text{S}]\text{methionine}$ 4 to 6 h after CCV infection (i.e. 20 to 22 h after infection with CRV) and radiolabeled proteins, separated by electrophoresis on 10% SDS-polyacrylamide gels, were visualized by autoradiography. Molecular weight markers are indicated in the left hand margin. To the right of the figure, a prominent CCV late protein is indicated by the large arrowhead, whereas a small arrowhead identifies a major early protein; to the left of the figure, several CRV proteins are indicated by filled squares

replication was only slightly affected and CRV protein synthesis was not detected, suggesting that CRV replication, and not simply virus attachment or entry, was required for the inhibition of CCV gene expression.

To further characterize CRV-mediated interference, infected cultures were examined 24 h after CCV infection by light microscopy. As shown in Fig. 3, CRV infection blocked the appearance of characteristic CCV syncytium in a multiplicity dependent fashion. At both high (MOI = $1.0 \text{ TCID}_{50} \text{ cell}^{-1}$, panel AH) and low (MOI = $0.1 \text{ TCID}_{50} \text{ cell}^{-1}$, panel AL) input levels, CRV infection itself had minimal impact on the short-term viability of CCO monolayers. In contrast, CCV infection at high (MOI = 8 pfu cell^{-1} , panel C1), intermediate (MOI = $0.8 \text{ pfu cell}^{-1}$, panel C2), or low (MOI = $0.08 \text{ pfu cell}^{-1}$, panel C3) MOI led to extensive syncytium formation. In co-infected cultures, especially at lower inputs of CCV (i.e. panels BH3 and BL3), prior infection with CRV blocked the formation of CCV-induced syncytia most likely by blocking CCV-specific protein syn-

Fig. 3. CRV infection inhibits CCV-induced cell fusion. Confluent monolayers of CCO cells were infected with CCV alone (the 3 left-most panels) at 8 (panel C1), 0.8 (panel C2), or 0.08 pfu cell^{-1} (panel C3), with CRV alone (MOI = $1 \text{ TCID}_{50} \text{ cell}^{-1}$, panel AH; and MOI = $0.1 \text{ TCID}_{50} \text{ cell}^{-1}$, panel AL), or with a mix of CRV and CCV. In the later case, CRV infection was at either 1 (panels BH2 and BH3) or $0.1 \text{ TCID}_{50} \text{ cell}^{-1}$ (panels BL2 and BL3) and was initiated 16 h prior to infection with CCV at either 0.8 (panels BH2 and BL2) or $0.08 \text{ pfu cell}^{-1}$ (panels BH3 and BL3). Forty-eight hours after CCV infection, monolayers were examined for CCV-induced cell fusion and photographed to document the extent of cytopathic effect. The large arrowhead in panel C1 identifies a multi-nucleated cell characteristic of CCV-induced fusion. The smaller arrowheads in panel C3 identify individual CCO cells that are not part of a syncytium

thesis. These results indicate that the reduction in CCV protein synthesis noted above in mixed infections was accompanied by a marked reduction in CCV-induced cell fusion.

To determine the effect of prior CRV infection on CCV virion formation, CCV titers in single and mixed infections were determined by plaque assay. As shown in Table 1, infection with CRV 16 h before superinfection with CCV resulted in markedly lower yields of the latter. Interestingly, whereas the 24 h yields of CCV were only about 2-fold different following infection at either intermediate (i.e. 0.8 pfu cell⁻¹) or low (i.e. 0.08 pfu cell⁻¹) MOIs, prior infection with CRV had a 10-fold greater inhibitory effect on the latter compared to the former. Furthermore, although simultaneous infection with CRV and CCV, or prior infection with UV-CRV or the S200 post-virion fraction at 16 h before CCV infection resulted in only a slight decrease in CCV protein synthesis (Fig. 2), a 70 to 90 % decrease in virus yields was detected by plaque assay (Table 1). Taken together these results indicate that prior infection with CRV markedly reduced the replication of CCV *in vitro*.

Induction of anti-viral activity by UV-CRV

Since reoviruses are good inducers of interferon, we attempted to determine whether the protection mediated by treatment with UV-CRV might be attributable

Table 1. Yield reduction assay: effect of CRV infection on CCV yields. At the indicated times prior to infection with CCV, cells were exposed to CRV (MOI = 0.9 TCID₅₀ cell⁻¹), UV-CRV, or the high speed supernatant fraction (S200) prepared from culture medium harvested from CRV-infected cells. Twenty-four hours after CCV infection, cultures were frozen and CCV yields determined by plaque assay on CCO cells. In a typical experiment, CCV yields were 4.7×10^7 pfu ml⁻¹ following infection at an MOI of 0.8 pfu cell⁻¹, and 7.6×10^7 pfu ml⁻¹ following infection at an MOI of 0.08 pfu cell⁻¹. % Control = (CCV yield following pretreatment ÷ CCV yield in untreated cells) × 100

Expt	Treatment 1	Time added	Treatment 2	% Control
1 ^a	CRV	-16 h	CCV	3.1 ± 2.5
	CRV	0 h	CCV	70 ± 7.1
	UV-CRV	-16 h	CCV	70 ± 18
	S200	-16 h	CCV	115 ± 47
	None	-	CCV	100
2 ^b	CRV	-16 h	CCV	0.3 ± 0.5
	CRV	0 h	CCV	12.3 ± 4.1
	UV-CRV	-16 h	CCV	30 ± 14
	S200	-16 h	CCV	34 ± 22
	None	-	CCV	100

^aIn Expt 1 CCV MOI was 0.8 pfu cell⁻¹
^bIn Expt 2 CCV MOI was 0.08 pfu cell⁻¹

to the *in situ* induction of an anti-viral factor (AVF) rather than to viral interference. CCO cells were infected with UV-CRV, and at 24 h intervals culture medium was harvested and tested for its ability to block CCV replication using a standard challenge assay. We observed (Fig. 4) that CCO cells synthesized (or secreted) an AVF following infection with UV-CRV. The titer of the AVF was higher in cultures infected with 1.0 versus 0.1 TCID₅₀ cell⁻¹ UV-CRV. For example on Day 2, the anti-viral titer in cells infected at the lower MOI was 160 IU ml⁻¹, whereas the titer in cells infected at the higher MOI was 2560 IU ml⁻¹. Maximal levels of AVF were seen within 24 to 48 h after infection and did not increase appreciably over the next 2 d. No anti-viral activity was detected in SCM harvested before CRV infection or from medium harvested from mock-infected cultures during the 4 d course of this experiment.

In a subsequent series of experiments the resistance of cells which synthesized (or secreted) anti-viral activity was monitored. CCO cells were infected with UV-CRV and culture medium harvested daily as described above. On the day media were harvested, the cultures were challenged with CCV and monitored 48 h later for the development of CPE and the production of infectious CCV. We observed that mock-treated CCO cells were readily susceptible to CCV infection and showed marked CPE within 48 h after low MOI infection with CCV, whereas UV-CRV-infected cultures showed little CPE (data not shown). Furthermore, the reduction of CPE correlated with a marked reduction in the ability of UV-CRV-infected CCO cells to support CCV replication. CCO cells treated 24 to 48 h earlier with UV-inactivated CRV then challenged with infectious CCV showed 48 h CCV yields ~5 % of those seen in mock-treated cells (average of 3 experiments). Surprisingly, protection was not long-lasting, and by 72 h after CCV infection UV-CRV-treated cultures showed marked CPE (data not shown).

Constitutive production of anti-viral activity by long-term, catfish lymphoid cell lines

In addition to the anti-viral activity generated in CCO cells following infection with UV-CRV, anti-viral activity was also constitutively present in SCM harvested from several long-term, catfish T cell-like and macrophage lines. Anti-viral titers as high as 6400 IU ml⁻¹ were detected in SCM from CF-59 (a T cell-like line). Moreover, the activity present in supernatants from 42TA cells (a macrophage line) was reduced 10-fold (from 1280 to 120 IU ml⁻¹) by boiling for 10 min, suggesting that the inhibitor was a heat-labile protein. Interestingly, anti-viral activity was not detected in

Fig. 4. Spent culture medium from CCO cells infected with UV-CRV protects CCO cells from infection with CCV. Four days after plating, CCO cells were mock-infected or infected with UV-CRV at a nominal MOI of 1 TCID₅₀ cell⁻¹ ('high MOI') or 0.1 TCID₅₀ cell⁻¹ ('low MOI'). At daily intervals the spent culture medium was removed, clarified by low speed centrifugation, and frozen until assay. Medium harvested at various days post infection, as well as spent culture medium (SCM) from CCO cells harvested prior to assay and DMEM/2% FCS (D2) were tested for anti-viral activity by challenge assay. The dilution factor (before addition of an equal volume of CCO cell suspension) is indicated above each column. The first 4 rows in the 'control' plate were mock-infected ('No CCV'), while all other wells on this and the remaining 3 plates were challenged with CCV ('+CCV')

SCM harvested from cloned B cell (e.g. 1G8) or fibroblast lines (F59 and CCO) not infected with CRV. In some experiments, growth medium from catfish peripheral blood lymphocytes (PBLs) proliferating in mixed lymphocyte culture (MLC) blocked CCV infection. Taken together, these results suggest that long-term catfish lymphoid cells may be an excellent source of fish cytokines.

DISCUSSION

The above work suggests that CRV interferes with CCV replication by 2 possible mechanisms: viral interference and induction of an AVF. Infection with CRV 16 h prior to infection with CCV blocked CCV as well as cellular macromolecular synthesis. Although the precise mechanism of inhibition of CCV by CRV has not yet been determined, it is likely that aquareoviruses, like their mammalian counterparts, block heterologous gene expression and regulate expression of their own products in a complex fashion (Nibert et al. 1996). In mammalian systems, reovirus infection leads to activation of DAI, a dsRNA-activated kinase, which specifically phosphorylates and inactivates the α subunit of eukaryotic initiation factor 2 (eIF-2) (Nilsen et al.

1982, Hovanessian 1991). Although phosphorylation of eIF-2 α leads to a global block in cellular protein synthesis, reovirus translation is maintained due to high, localized concentrations of the reovirus protein δ 3, which binds dsRNA and blocks activation of DAI (Schmechel et al. 1997). In cells infected with CRV alone translational shutoff results in the gradual replacement of cellular gene expression by reovirus, whereas in cells co-infected with CRV and CCV, CRV infection appears to suppress both host and heterologous virus gene expression. Inspection of Fig. 2 supports this view and shows that in CRV-infected cells reovirus proteins are clearly seen against a background of diminished cellular protein synthesis. Furthermore, the marked inhibition of cellular protein synthesis following CRV infection may also explain why only low levels of the AVF are synthesized in productively-infected cells, i.e. inhibition of cellular protein synthesis prevents the accumulation of high levels of the AVF.

Viral interference has been reported following infection of fish or fish cell lines with homologous or heterologous viruses (Nicholson & Dunn 1974, de Kinkelin et al. 1992). Hedrick et al. (1994) showed that pre-exposure of rainbow trout to avirulent cutthroat trout virus (CTV) reduced mortality associated with subsequent infection by infectious hematopoietic necrosis

virus (IHNV). The mechanism(s) of resistance induced by CTV was unknown, but CTV was found to be a potent inducer of interferon-like activity. In a second study, LaPatra et al. (1995) showed that prior infection of rainbow trout with avirulent chum salmon reovirus resulted in increased survival when the fish were challenged with IHNV over an 8 wk period. As with the previous study, the mechanism of protection was unknown although it was suggested that protection may involve interferon induction and/or stimulation of macrophage or natural killer cell functions.

Following exposure to UV-CRV, anti-viral activity was induced and reached maximal levels within 24 to 48 h (Fig. 4). The kinetics of AVF induction in CCO cells cultured at 27°C are nearly as rapid as interferon induction in mammalian cells incubated at 37°C, and are consistent with the proposed role of interferon as a first line of defense following virus infection. Protection afforded by catfish AVF was incomplete and could be overcome by increasing the input of challenge virus. Furthermore, although cultures producing AVF were protected from CCV challenge for ~48 h, during the next 24 h 'residual' virus replicated and caused extensive CPE. The basis for this phenomenon is not known but may reflect waning of the anti-viral state. Consistent with this observation are findings in other systems where the protection mediated by interferon could only be demonstrated when the input level of challenge virus was low (Pusateri et al. 1980). Alternatively, it is possible that the system chosen to monitor anti-viral activity (i.e. CCV infection of CCO cells) is not optimal for assessing activity. CCV, as is the case with other large DNA viruses, may encode proteins which antagonize interferon action, and CCO cells may not be as sensitive to the induction of anti-viral proteins as other cell types. Furthermore, marked variation is seen among piscine herpesviruses in their sensitivity to interferon (Frerichs 1989).

In addition to induction with UV-CRV, anti-viral activity was constitutively present in several long-term, catfish lymphoid cell lines. Levels of anti-viral activity, similar to those seen following UV-CRV infection, were detected in supernatants harvested from T cell-like (CF-59) and macrophage (42TA) cell lines. In contrast, B cell clones (1G8) and fibroblast cell lines (F59, CCO) did not constitutively produce AVF. Previous work showed that newly established T cell and macrophage lines were non-permissive for CCV replication (Chinchar et al. 1993). In view of the above findings, this defect may be due to the production of an AVF by these cultures. Interestingly, as T cell/macrophage lines were maintained in culture, they gradually became more permissive to CCV infection, perhaps because they lost their ability to synthesize 'non-essential' proteins (e.g. cytokines). Consistent with this

suggestion, long-term catfish lymphoid cell lines, which initially secreted compounds functionally homologous to IL-2 and IL-4, lost their ability to synthesize and/or secrete these 'luxury' proteins with continued *in vitro* passage (C. Luft & N. W. Miller pers. comm.).

Although an AVF has been detected in catfish cells by its biological activity, its identity has not yet been determined. Furthermore, it is not clear whether the anti-viral activity seen in CCO cultures after infection with UV-CRV and the activity detected constitutively in long-term lymphoid cell lines are identical. It is possible that the activity induced by UV-CRV in fibroblasts is type I interferon (interferon α/β), whereas the activity seen in T cells and macrophages is related to type II interferon (interferon γ). Cytokine activity has been detected in catfish lymphoid cell cultures following mitogen stimulation (Caspi & Avtalion 1984, Grondel & Harmsen 1984, Clem et al. 1985, Miller et al. 1985, Lin et al. 1992, Clem et al. 1996). Furthermore, rapidly proliferating MLC cultures contain increased numbers of activated natural killer and/or T cells (Stuge et al. 1997) and likely synthesize interferon γ and a variety of other cytokines. Consistent with these results, Graham & Secombes (1990) found that an interferon γ -like activity was secreted by trout leukocytes following mitogen stimulation. However, attempts in our laboratory to detect mRNA encoding type I interferon or tumor necrosis factor (TNF) α in long-term catfish lymphoid cell lines by Northern blot analysis using mammalian probes for TNF α , interferon α , and interferon β or by reverse transcription polymerase chain reaction (RT-PCR) were not successful. These results suggest either the absence of interferon α/β and TNF α transcripts in these cells or that the piscine genes encoding these proteins may be only distantly related to their mammalian homologs. The latter view is strengthened by recent studies showing that chicken interferon genes share little sequence identity with their mammalian counterparts (Sekellick et al. 1994, Lowenthal et al. 1995). Thus, while the AVF may be piscine interferon, definitive proof awaits additional physio-chemical and genetic confirmation.

In summary, the work described herein provides evidence of virus-mediated interference in catfish, and is the first report of an interferon-like, anti-viral activity in this species. The ability of CCO cells to synthesize detectable levels of an interferon-like factor following infection with UV-CRV, coupled with the ability of several lymphoid cell lines to synthesize AVF constitutively, suggests that catfish may be an excellent system in which to study piscine interferon-like activity and explore the evolutionary origins of this important component of innate immunity. This system may provide the key which will allow researchers to progress beyond the mere description of interferon-like activity in fish and definitively identify these important cytokines.

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