Molecular Cloning and Expression Analysis of Lysozyme C and MHC class I from Crucian Carp *Carassius auratus gibelio* in Response to Cyprinid Herpesvirus 2 Infection

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ABSTRACT—*MHC class I (MHC I)* and *Lysozyme C (LYZ C)* play important roles in disease resistance in fish species, and are thought to participate in anti-viral innate immunity. Previously, after Cyprinid herpesvirus 2 (CyHV-2) infection, *MHC I* and *LYZ C* were found to be expressed in both moribund (acute infection) and survivor (chronic infection) fish based on suppression subtractive hybridization (SSH) analysis. Herein, the complete open reading frames (ORFs) of *MHC I* and *LYZ C* were cloned from crucian carp (*Carassius auratus gibelio*). The full-length cDNAs of these two genes contained ORFs of 1,038 and 438 bp, and encoded polypeptides of 345 and 145 amino acids, respectively. Phylogenetic analysis indicated that *MHC I* and *LYZ C* were most closely related to the common carp. We found that the two genes were expressed at low levels in all tissues examined in healthy crucian carp. The mRNA expression levels of *MHC I* and *LYZ C* in crucian carp kidney tissues was monitored at different time points for 72 h after CyHV-2 infection using real-time RT PCR analysis. During CyHV-2 infection experiments, peak levels of *MHC I* were observed at 72 hpi (5.55-fold, $P < 0.01$), whereas 48 hpi (3.87-fold, $P < 0.01$) was the peak expression time point for *LYZ C*. By analysis of samples from both acute and chronic infection, the expression levels of *MHC I* and *LYZ C* were up-regulated in comparison to uninfected fish. These data suggested that *MHC I* and *LYZ C* are involved in cellular response to CyHV-2 infection. The discovery of *LYZ C* induced by CyHV-2 in crucian carp suggests its potential role in fish innate immunity against viral pathogen as *MHC I*.

Key words: *Carassius auratus gibelio*, crucian carp, innate immunity, *MHC class I*, *Lysozyme C*

The crucian carp *Carassius auratus gibelio* is one of the major aquaculture species in China and has become the fourth most important farmed freshwater fish (Wu *et al.*, 2013). To date, viral infections in crucian carp have caused significant economic losses. Since 2011, massive mortality of crucian carp has been observed in many aquatic breeding ponds in northern Jiangsu province, eastern China. Cyprinid herpesvirus 2 (CyHV-2), also known as herpesviral hematopoietic necrosis (HVHN), was detected and confirmed to be a pathogen (Wang *et al.*, 2012; Wu *et al.*, 2013). The clinical signs of disease included pink ascites in the abdominal cavity, lethargy, and lack of appetite, and the internal gross pathology of the disease included bleeding and pale gills, and swollen spleen and kidneys. The process developed rapidly and the mortality rates reached 90–100% (Xu *et al.*, 2013).

Many immune-related genes that are involved in

CyHV-2 infection in crucian carp have been identified by suppression subtractive hybridization (SSH) analysis. We previously identified 42 different immune-related genes from moribund (acute infection) and survivor (chronic infection) fish libraries. The moribund library included *SAA*, *Mtap*, *IL-10R*, *TUBA2*, *Nfkbiab*, *ccC7*, *PNP5a*, *intelectin*, and *IL-11*. The survivor library included *granulin*, *Mta3*, *ANP 32A*, *eno1a*, *pyroxd2*, *Hsc70kDa*, *tuba8l2*, *SET B*, *CCT*, *CXCR4*, *NP*, *IAP*, *mibp*, *CALR*, *Ptpn6*, *pmt*, *lamn*, *Cts L*, *Cts C*, *capg*, *rac2*, *tubulin*, *Cts B*, *eml2*, *cxc112*, *Rab-12*, *Tms b-11*, *nfkbiaa*, *keratin8*, *dusp1*, and *MPO*. Interestingly, the major histocompatibility complex (*MHC*) class I (*MHC I*; GenBank accession no. KC969194.1) and *Lysozyme C* (*LYZ C*; GenBank accession no. KF417503.1) genes were present in both SSH libraries (Xu *et al.*, 2014). Little is known about the *MHC I* and *LYZ C* genes in the anti-viral response in crucian carp. Therefore, in this study, we focused on characterizing the basic roles of *MHC I* and *LYZ C* in anti-viral innate immunity.

The innate immune system is the primary host

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response that serves as the first line of defense against pathogens during the first few hours after infection. It also acts to slow down pathogen replication or avert microbial attack. *MHC I* and *LYZ C* encode two important innate immune molecules. *MHC I* plays an important role in alerting the immune system to cells infected with a virus. *MHC I* molecules are primarily associated with defense against intracellular pathogens, and present peptides derived from degraded intracellular pathogens to cytotoxic CD8⁺ T cells, which can kill infected target cells (Fischer *et al.*, 2006). The *MHC I* antigen presentation pathway is initiated by the proteolysis of ubiquitinated proteins in the proteasome, generating T cell epitopes, which are antigenic peptides that are capable of eliciting an immune response (Goldberg *et al.*, 2002). *MHC class I* complexes are expressed on the surface of all nucleated cells (except sperm cells and some neurons), and present peptide fragments that are derived from intracellular pathogens (Hewitt, 2003; Pierny and Oliver, 2006).

Lysozyme is an important molecule in innate immunity, as it mediates protection against microbial invasion. It is involved in various defense mechanisms, including immunomodulatory, anti-bacterial, anti-inflammatory, anti-tumor, and anti-viral activities (Kokoshis *et al.*, 1978; Lee-Huang *et al.*, 1999; Salton, 1957; Sava *et al.*, 1988). C-type lysozymes have been identified in fish, including orange-spotted grouper *Epinephelus coioides* (Wei *et al.*, 2012), grass carp *Ctenopharyngodon idellus* (Ye *et al.*, 2010), Japanese flounder *Paralichthys olivaceus* (Minagawa *et al.*, 2001), Turbot *Scophthalmus maximus* (Jimenez-Cantizano *et al.*, 2008), and Senegalese sole *Solea senegalensis* (Fernández-Trujillo *et al.*, 2008). In fish, a lysozyme enzyme with antibiotic properties can be released by some leucocytes, which mainly include neutrophils, monocytes, and to a lesser degree macrophages (Lie *et al.*, 1989). The close association of lysozyme with the cellular immune system could indicate that this enzyme contributes to defence against infectious diseases (Saurabh and Sahoo, 2008).

Here, we report our characterization of the *MHC I* and *LYZ C* genes in crucian carp, focusing on responses to CyHV-2 infection. We studied the cellular response by measuring *MHC I* and *LYZ C* transcript levels during both acute and chronic infection, and also investigated the basic role of these molecules in anti-viral innate immunity.

Materials and Methods

Fish and virus challenge

Crucian carp *Carassius auratus gibelio* weighing ~200 g were obtained from a local farm and acclimatized to laboratory conditions for two weeks in the Shanghai Ocean University fish-breeding farm. The

CyHV-2 strain was isolated from diseased crucian carp *Carassius auratus gibelio* samples that were cultured in Sheyang City, Jiangsu Province in May 2012. The purified virus solution and LD₅₀ dose have been described previously (LD₅₀ dose = 1×10^6 particles/mL) (Xu *et al.*, 2014). For viral infections, fish were intraperitoneally injected with 1 mL virus at a concentration of 10^6 viral particles/mL. Fish were maintained at a controlled water temperature between 20–23°C. A mock-infected control group was administered PBS alone.

Sample collection

To investigate the tissue distribution of *MHC I* and *LYZ C* mRNA in healthy crucian carp, we collected liver, kidney, spleen, heart, gill, and muscle tissues from three healthy fish.

To examine the mRNA expression levels of *MHC I* and *LYZ C* genes after CyHV-2 infection within 72 hours post-injection (hpi). Kidney tissues were collected at 0, 6, 12, 24, 48, and 72 hpi. Three individual fish were sampled for each group at each time point.

To characterize the mRNA expression levels of *MHC I* and *LYZ C* in moribund and surviving fish after challenge with CyHV-2. Fish were observed continuously for clinical signs, including bleeding and pale gills, gasping movements in shallow water, and pink ascites in the abdominal cavity (data not shown). Mortality was only detected at 72 h post viral challenge. Samples collected at 72 hpi were defined as moribund samples. The moribund samples from viral infection was confirmed by PCR. No further mortality was observed at 168 h post-viral challenge. Samples collected at 168 hpi were defined as surviving samples. Kidney tissues were collected from four individuals per group. Three biological replicates per fish were collected and stored at -80°C until later use.

RNA extraction and cDNA synthesis

Total RNA was isolated from the kidney tissue using TRIzol (Invitrogen) following the manufacturer's instructions. The purity of the extracted RNA was determined by the OD_{260nm}/OD_{280nm} ratio, with expected values between 1.8 and 2.0. All RNA samples were treated with RNase-free DNaseI (Takara) to remove residual genomic DNA before it was reverse transcribed into cDNA using random hexamer primers and MMLV Reverse Transcriptase (Takara) according to the manufacturer's instructions.

Cloning full-length cDNAs of MHC I and LYZ C

Gene-specific primer pairs were designed for *MHC I* and *LYZ C* based on partial gene sequences (*MHC I*, GenBank accession no. KC969194.1; and *LYZ C*, GenBank accession no. KF417503.1) using the Primer Premier 5 program. These oligos were used with the adaptor primers UPM and NUP to amplify the genes

from crucian carp cDNA (Table 1). The full-length sequence, 5'-untranslated regions (5'-UTRs), and 3'-untranslated regions (3'-UTRs) for the *MHC I* and *LYZ C* genes were obtained using the SMARTer™ RACE cDNA Amplification kit (Clontech Laboratories, Inc.) to synthesize first-stand cDNA according to the manufacturer's instructions. The PCR conditions were: 94°C for 1 min, 25 cycles of 94°C for 30 s, 68°C for 30 s, and 72°C for 2 min, and a final extension at 72°C for 10 min. The PCR products were gel-purified using the Wizard® SV Gel and PCR Clean-Up System (Promega) before being cloned into the pMD 19-T vector (Takara). The pMD 19-T vector with each full-length gene was transformed into *Escherichia coli* DH5α competent cells, plated on an LB-agar Petri-dish, and incubated overnight at 37°C. Positive colonies containing inserts of the expected size were screened by colony PCR. Three positive clones were picked for commercial sequencing (Shenggong Biotech).

Multiple sequence alignment and phylogenetic analysis

The BLAST program from the National Center for Biotechnology Information (NCBI) was used to search for homologous sequences in GenBank. The open reading frames (ORFs) for *MHC I* and *LYZ C* were determined using ORF Finder (<http://www.ncbi.nlm.nih.gov/projects/gorf/>). DNASTar software was used to predict the protein amino acid sequences. Multiple sequence alignments were generated with DNAMAN software (Lynnon Biosoft). Neighbor-joining phylogenetic analysis was performed using the predicted amino acid sequence. The phylogenetic tree was generated based on a ClustalW alignment using the MEGA5 program. Robustness of nodes was ensured using 1000 bootstrap iterations.

Real-time RT-PCR analysis

Real-time RT-PCR was used to quantify gene expression in kidney tissues after viral infection. First-strand cDNA was used as a template for the gene-specific primers shown in Table 1. *β-ACTIN* was used as a reference gene for each sample. Real-time RT-PCR was carried out in a 20-μL reaction volume containing 1 μL cDNA sample, 7 μL nuclease-free water, 10 μL 2× SsoAdvanced™ SYBR Green Supermix (Bio-Rad), and 1 μL of each gene specific primer (10 μM). The *MHC I* and *LYZ C* mRNA real-time RT-PCR cycling conditions were: 95°C for 30 s, 40 cycles of 95°C for 5 s, 60°C for 30 s, 95°C for 1 min, and 65°C for 1 min, followed by a dissociation curve analysis to verify the amplification of a single product; the curve ramped from 65°C to 95°C in 0.5°C increments at 5 s intervals. Real-time RT-PCR was performed using a CFX96™ Real-time PCR Detection System (Bio-Rad). After the PCR program was completed, the threshold cycle (CT) value was determined using the manual setting on the CFX Manager 2.1 software (Bio-Rad) and exported into a Microsoft Excel Sheet for subsequent data analyses in the SPSS software package. For tissue distribution analysis, the $2^{C_{T\text{reference}}/2^{C_{T\text{target}}}}$ ratio of the target gene vs. reference gene (*β-ACTIN*) was calculated as previously described (Marjara *et al.*, 2010). The relative expression levels of *MHC I* and *LYZ C* after CyHV-2 infection were calculated as the relative fold-changes compared to *β-ACTIN* by the $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen, 2001). Expression data obtained from independent biological replicates were subjected to one-way analysis of variance (one-way ANOVA), followed by a paired-samples *t*-test. *P*-values < 0.05 were considered to indicate statistically significant differences.

Table 1. Primers used for the sequencing of full-length transcripts and real-time RT-PCR

Primer name	Sequence (5'–3')	Annealing Temperature (°C)	Primer information
5'-RACE-MHC I	GAAGAAGTTCACCAACCTCCACATCCTC	68	5'-RACE
3'-RACE-MHC I	CAACAAATGGGCTCTACCAGGATGC	68	3'-RACE
5'-RACE-LYZ C	ATCCTTTTACCCAGCGTGTCTATCT	68	5'-RACE
3'-RACE-LYZ C	CAGGCTGGGTCGCTGTGATGTCGC	68	3'-RACE
Universal Primer A Mix (UPM)	Long: CTAATACGACTCACTATAGGGCA AGCAGTGG TATCA ACGCAGAGT		RACE
Nested Universal Primer A (NUP)	Short: CTAATACGACTCACTATAGGGC AAGCAGTGGTATCAACGCAGAGT		RACE
MHC I	Forward: CTCATCTCCAGTCGTGTGTCA Reward: AAAGGTCCCCTCATCATTAGG	60	qRT-PCR
LYZ C	Forward: ACTTGATGGCTTTGAGGGATT Reward: TTACACTGGTTCTTTCCACCTG	60	qRT-PCR
<i>β-ACTIN</i> (GenBank accession no. AB039726.2)	Forward: CACTGTGCCCCTCTACGAG Reward: CCATCTCCTGCTCGAAGTC	60	qRT-PCR

Fig. 1. The full-length cDNA of *MHC I* from crucian carp. (A) The nucleotide sequence along with the predicted amino acid sequences; numbers on the left hand side indicate the sequence position. The box indicates the start codon (ATG) and the “*” marks the stop codon (TGA). Underlining indicates motifs associated with mRNA instability (ATTTA), and a dashed line indicates a polyadenylation signal (AATAAA). (B) A multiple alignment of *MHC I* amino acid sequences from different organisms. Missing amino acids are marked by dots, identical amino acids (100%) are indicated in yellow, similar amino acids ($\geq 75\%$) are shown in orange, and somewhat similar amino acids ($\geq 50\%$) are shown in grey. Numbers on the right indicate the amino acid position in the corresponding species.

Fig. 2. The full-length cDNA of *LYZ C* from crucian carp. (A) The nucleotide sequence along with the predicted amino acid sequences; numbers on the left hand side indicate the sequence position. The box indicates the start codon (ATG) and the “*” marks the stop codon (TAA). Underlining indicates motifs associated with mRNA instability (ATTTA), and a dashed line indicates a polyadenylation signal (ATTAAA). (B) A multiple alignment of *LYZ C* amino acid sequences from different organisms. Missing amino acids are marked by dots, identical amino acids (100%) are indicated in yellow, similar amino acids ($\geq 75\%$) are shown in orange, and somewhat similar amino acids ($\geq 50\%$) are shown in grey. Numbers on the right indicate the amino acid position in the corresponding species.

Fig. 3. Phylogenetic analysis of *MHC I* (A) and *LYZ C* (B) with all available animal sequences using the Neighbor-Joining method was conducted based on a ClustalW alignment with the MEGA5 program. Numbers near each node represent bootstrap values. Scale bar, the number of substitutions per site. The following *MHC I* sequences are available from GenBank: common carp (*Cyprinus carpio*), HM372879.1; grass carp (*Ctenopharyngodon idella*), AY391782.2; zebrafish (*Danio rerio*), NM131704.1; mouse (*Mus musculus*), NM008209.4; cattle (*Bos Taurus*), NM001114855.1; chimpanzee (*Pan troglodytes*), NM001012439.2; and human (*Homo sapiens*), L38504.1. The *LYZ C* sequences are as follows: common carp (*Cyprinus carpio*), AB027305.1; grass carp (*Ctenopharyngodon idella*), EU835654.1; zebrafish (*Danio rerio*), NM139180.1; black tiger shrimp (*Penaeus monodon*), JX912160.1; cattle (*Bos Taurus*), M95099.1; house fly (*Musca domestica*), EU719198.1; western gorilla (*Gorilla gorilla*), NM001279662.1; and human (*Homo sapiens*), M21119.1.

Fig. 4. Tissue expression of the crucian carp *MHC I* (A) and *LYZ C* (B) genes. Expression was determined using real-time RT PCR in three healthy fish. Each sample was measured in biological triplicates. β -ACTIN was used as an internal reference gene.

Fig. 5. Quantitative gene expression profiles of *MHC I* (A) and *LYZ C* (B) after the challenge with CyHV-2 at 6, 12, 24, 48, and 72 h post-infection using real-time RT-PCR analysis. Controls were injected with PBS. β -ACTIN was used as an internal reference gene. Error bars indicate standard error (N = 3). Asterisks indicate significant differences between the CyHV-2 infected and control groups detected with a paired-samples *t*-test (* $P < 0.05$, ** $P < 0.01$).

Fig. 6. The gene expression levels of *MHC I* (A) and *LYZ C* (B) in moribund (M) and surviving (S) crucian carp after CyHV-2 infection were compared with the control groups. For viral challenges, fish were infected with 1 mL containing 10^6 CyHV-2 particles intraperitoneally, and control groups were infected with 1 mL PBS alone ($N = 4$ per group). The mRNA expression levels of *MHC I* and *LYZ C* were measured in the kidney of moribund and survivor fish. β -ACTIN was used as an internal reference gene. Error bars indicate standard error ($N = 3$). Asterisks indicate significant differences between the CyHV-2-infected and control groups detected with a paired-samples *t*-test (* $P < 0.05$, ** $P < 0.01$).

liver, spleen, gill, muscle, heart, and kidney tissues of healthy crucian carp and was normalized using β -ACTIN expression levels. The *MHC I* and *LYZ C* mRNA transcripts were expressed at very low levels in all the tissues examined, although comparatively higher mRNA expression levels of *MHC I* were detected in muscle tissues (Fig. 4A), whereas *LYZ C* was more abundant in kidney tissues (Fig. 4B).

The mRNA expression levels of *MHC I* and *LYZ C* genes 72 h after CyHV-2 infection

We determined the mRNA expression patterns of *MHC I* and *LYZ C* in crucian carp kidney tissues after CyHV-2 infection using real-time RT PCR analysis at different time points (Fig. 5). The transcript for *MHC I* was significantly up-regulated at 12 h post-infection (hpi; 2.21-fold, $P < 0.05$), before declining at 24 and 48 hpi (1.58- and 1.42-fold, respectively, $P > 0.05$), and finally showing a peak expression level at 72 hpi (5.55-fold, $P < 0.01$; Fig. 5A). For *LYZ C*, the expression level was increased at 24 hpi (1.96-fold, $P < 0.05$) and reached its peak at 48 hpi (3.87-fold, $P < 0.01$), then decreased to 1.84-fold at 72 hpi ($P > 0.05$; Fig. 5B).

A comparison of the mRNA expression levels of *MHC I* and *LYZ C* in acute versus chronic CyHV-2 infection

Previously (Xu *et al.*, 2014), we detected *MHC I* and *LYZ C* gene transcripts in both moribund (acute

infection) and survivor (chronic infection) SSH libraries after CyHV-2 infection. To confirm our SSH results, we examined the expression levels of *MHC I* and *LYZ C* in moribund and surviving fish after viral challenge. In kidney tissues, *MHC I* mRNA expression levels in moribund and surviving samples were increased 3.17- and 3.05-fold ($P < 0.01$) respectively (4 fish per group; Fig. 6A). Similarly, the expression levels of *LYZ C* in moribund and surviving fish, were significantly up regulated by 2.32- and 2.56-fold ($P < 0.05$), respectively (Fig. 6B). These results confirmed that the up-regulation of *MHC I* and *LYZ C* could be detected in both acute and chronic infection after CyHV-2 challenge in crucian carp.

Discussion

In our study presented here, the relative expression levels of *MHC I* and *LYZ C* in healthy crucian carp were very low in all tissues tested. Comparably higher mRNA levels of *MHC I* were detected in muscle. By contrast, high levels of each *MHC class I and II* in stickleback *Gasterosteus aculeatus* could be observed in gills and spleen by western blotting (Scharsack *et al.*, 2007). Crucian carp showed comparably higher levels of *LYZ C* expression in kidney tissues, consistent with the highest lysozyme expression in rainbow trout, which also was most abundant in kidney tissues (Lie *et al.*, 1989). Moreover, the c-type lysozyme gene (*Ec-lysC*)

transcript levels of orange-spotted grouper *Epinephelus coioides* were most abundant in the head kidney (Wei *et al.*, 2012) suggested that *LYZ C* was expressed mainly in the immunity related tissues and might be the main function for the defense against pathogens in fish. Protein levels could also be detected using a lysozyme-specific monoclonal antibody (mAb M24-2), which showed intracellular lysozyme protein in head kidney cells of the mummichog (*Fundulus heteroclitus*) (Marsh and Rice, 2010).

To the best of our knowledge, this is the first time that the effect of CyHV-2 infection on the expression of *MHC I* and *LYZ C* have been examined in crucian carp *Carassius auratus gibelio*. The moribund fish had experienced acute infection, whereas the surviving fish had undergone chronic infection. In this study, after CyHV-2 infection, *MHC I* showed the highest up-regulation at 72 hpi. Similar to HepG2 human hepatocytes, 24 h after infection with Dengue virus (DV-2), *MHC I* increased 2.25-fold compared to mock-infected cells (Othman *et al.*, 2012). In Atlantic salmon, infection with the early infectious salmon anaemia virus (ISAV) induced a rapid type I and II IFN response in head kidney, spleen, and heart. Additionally, it induced the expression of *MHC I* (Jorgensen *et al.*, 2007). After challenge with ISAV in Atlantic salmon, *MHC I* and *II* showed highly significant associations for resistance to infectious diseases (Grimholt *et al.*, 2003). Furthermore, the *MHC I* Sasa-B-04 allele was highly associated with resistance to infectious hematopoietic necrosis virus (IHNV) in Atlantic salmon ($P < 0.01$) (Miller *et al.*, 2004). The expression levels of MHC isoforms were up-regulated upon either *in vivo* and *in vitro* stimulation with *Andrias davidianus* ranavirus (ADRV) in the Chinese giant salamander (Zhu *et al.*, 2014). By contrast, Herpes simplex type-1 infection significantly reduced the expression level of *MHC I* on the cell surface of glioma cells (Song *et al.*, 1999). *MHC* genes were expressed after viral infection in some important species, such as crucian carp, human, Atlantic salmon, and Chinese giant salamander. The expression levels of *MHC I* from both acute and chronic infection were significantly up-regulate in comparison to uninfected fish, it may be because the *MHC I* pathway processes intracellular viral proteins when viruses are present in infected cells, and then displays them on the cell surface. It can alert and stimulate cellular immune responses, which eventually kills the infected cell. To escape this cellular immune response, many viruses down-regulate the cell surface molecules encoded by the *MHC* genes (Othman *et al.*, 2012). Moreover, *MHC I* complexes are also associated with natural killer (NK) cells, which provide the first line of defence against intracellular pathogens (Unanue, 1997) by mediating cytotoxicity. NK cell cytotoxic activity is partially controlled by *MHC* receptors, which are one of the main elements of the

acquired immune system (Valés-Gómez *et al.*, 2000).

The interactions of lysozyme with DNA molecules might interfere with DNA replication, modulate gene expression, and block bacterial and viral infections (Lin *et al.*, 2009). In our CyHV-2 infection experiment, expression levels of *LYZ C* were significantly up-regulated by 24 hpi, and peak levels were measured at 48 hpi. Similar to *Ec-lysC* of the orange-spotted grouper in the head kidney, *Ec-lysC* transcripts were most abundant 24 h after SGIV infection and, in grouper cells, overexpression of *Ec-lysC* delayed the occurrence of CPE and significantly inhibited viral gene transcription (Wei *et al.*, 2012). Following CyHV-2 infection, *LYZ C* was found in both moribund and surviving fish. This could be because *LYZ C* is involved in restricted antiviral activity by released from leucocytes (Jolles and Jolles, 1984; Saurabh and Sahoo, 2008). A lysozyme dimer (KLP-602) stimulated the cellular and humoral defence mechanisms of rainbow trout. Additionally, the mortality of the lysozyme-treated groups was significantly lower compared to the control group after IPNV infection (Siwicki *et al.*, 1998). Similar findings were made in blue shrimp *Litopenaeus stylirostris*, as *Lslzm* protein increased resistance to WSSV, with infection survival rates reaching 70–80%, compared with infected shrimp injected with PBS alone (Mai and Wang, 2010). Lysozyme also blocked WSSV infection in blue shrimp *Litopenaeus stylirostris* and modulated the cellular and humoral defense mechanisms after viral infection (Mai and Wang, 2010). Moreover, lysozyme from chicken egg white, human milk, and human neutrophils exert anti-viral activities and can block HIV-1 replication in human cells (Lee-Huang *et al.*, 1999). The molecular mechanism of antiviral effect may be caused by degradation of viral polysaccharides (Lee-Huang *et al.*, 1999). Furthermore, lysozyme works with another anti-HIV protein present in urine, which also acts to prevent the virus from replicating (Singh *et al.*, 2005). Finally, lysozyme in the human placenta can reduce the adsorption of ectromelia virus and could play important protective roles during pregnancy (Arimura, 1973).

In conclusion, we cloned and characterized *MHC class I* and *C-type lysozyme* genes from the crucian carp. Our data indicated that these two genes were upregulated after CyHV-2 challenge during both acute and chronic infection. These findings suggest that *MHC I* and *LYZ C* are involved in the cellular response to CyHV-2. The discovery that *LYZ C* can be induced by CyHV-2 in the crucian carp suggests it has a basic role in fish innate immunity against viral pathogen as *MHC I*.

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