Vol. 73: 113–122, 2006 | DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org | Published December 14

Differential gene expression profile in spleen of mandarin fish *Siniperca chuatsi* infected with ISKNV, derived from suppression subtractive hybridization

Wei He[1,†], Zhi-Xin Yin[1,†], You Li[1], Wei-Li Huo[1], Hao-Ji Guan[1], Shao-Ping Weng[1], Siu-Ming Chan[2], Jian-Guo He[1,*]

[1]State Key Laboratory for Biocontrol, School of Life Sciences, Zhongshan University, 135 Xingang Road West, Guangzhou 510275, PR China

[2]Department of Zoology, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, PR China

ABSTRACT: To study the interaction between an invading virus and its host, we investigated differential gene expression in mandarin fish *Siniperca chuatsi* experimentally infected with infectious spleen and kidney necrosis virus (ISKNV). Subtractive cDNA libraries were constructed by suppression subtractive hybridization (SSH) from spleens of mock- and ISKNV-infected fish. Both forward- and reverse-subtracted libraries were generated. In the forward library, genes of the ubiquitin–proteasome proteolytic pathway, defense-related genes, a cytoskeletal protein gene, an apoptosis-related gene encoding inhibitor of apoptosis protein and JFC/EBPb cDNA for CAAT/Enhancer binding protein beta were up-regulated after infection. In the reverse library, genes that encoded CD59/neurotoxin/Ly-6-like protein, carboxypeptidase A2, and goose-type lysozyme were down-regulated. Some of these genes were analyzed by reverse transcription-polymerase chain reaction to confirm their differential expression as a result of virus infection. The results of this study may contribute to our understanding of fish innate immune response to ISKNV.

KEY WORDS: Infectious spleen and kidney necrosis virus · ISKNV · *Siniperca chuatsi* · Suppression subtractive hybridization · SSH · Ubiquitin–proteasome proteolytic pathway · Apoptosis

INTRODUCTION

Iridoviruses are large (~120 to 200 nm) DNA viruses that can infect invertebrates and poikilothermic vertebrates, including insects, fishes, amphibians and reptiles (Williams 1996). The family *Iridoviridae* has been divided into 5 genera, including *Iridovirus, Chloriridovirus, Ranavirus, Lymphocystisvirus* and *Megalocystivirus* (http://phene.cpmc.columbia.edu/Ictv/index.htm). In recent years, megalocystiviruses have attracted much attention because most of them can cause serious systemic diseases in a wide-range of economically important freshwater and marine fish species worldwide. Fish affected by the megalocystivirus include red sea bream *Pagrus major* (Inouye et al. 1992), sea bass *Lateolabrax* sp. (Nakajima & Sorimachi 1995), brown-spot grouper *Epinephelus tauvina* (Chua et al. 1994), malabar grouper *E. malabaricus* (Danayadol et al. 1996), angelfish *Pterophyllum scalare* (Rodge et al. 1997), grouper *Epinephelus* sp. (Chou et al. 1998), tilapia *Oreochromis niloticus* (McGrogan et al. 1998), mandarin fish *Siniperca chuatsi* (He et al. 2000), African lampeye *Aplocheilichthys normani* (Sudthongkong et al. 2002a), dwarf gourami *Colisa lalia* (Sudthongkong et al. 2002b), red drum *Sciaenop socellata* (Weng et al. 2002), rock bream *Oplegnathus fasciatus* (Jung & Oh 2000), large yellow croaker *Larimichthys croce* (Chen et al. 2003) and turbot

*Corresponding author. Email: lsbrc05@zsu.edu.cn
†These authors contributed equally to this work

Scophthalmus maximus (Shi et al. 2004). In a recent investigation of host range performed in the South China Sea, 13 cultured and 39 wild marine fish species were confirmed hosts of the megalocystiviruses (Wang et al. 2006).

Infectious spleen and kidney neurosis virus (ISKNV) is the type species of the genus *Megalocystivirus*. It can infect the mandarin fish *Siniperca chuatsi* Basilewsky, which will develop serious systemic disease (He et al. 2002). The virus infection is capable of causing severe epizootics resulting in mass mortalities. The mandarin fish is the main farmed fish species in China with an annual output reaching 140 000 tons and valued at 1 billion US dollars in 2003. In the last decade, the spread of ISKNV has resulted in significant economic losses for many fish farms (He et al. 1998, 2000). Therefore, preventing and controlling the spread of megalocystivirus has become a priority to the fish industry.

An infectious disease is the manifestation of a dynamic series of events that occur between the host and pathogen, which are defined by the interaction of pathogen-expressed virulence factors and the surveillance and defense systems of the host. Presently, much remains to be elucidated about host–megalocystivirus interactions at the molecular level. In this study, a sensitive PCR-based subtraction approach, suppression subtractive hybridization (SSH) (Diatchenko et al. 1996) was adopted to isolate genes up- or down-regulated in ISKNV-infected mandarin fish and identify their expression profile. cDNAs from the forward and reverse subtracted libraries were sequenced and analyzed. We identified some genes in the spleen of the mandarin fish that may be involved in the defense against ISKNV. These results will help to understand the infectious mechanism of the virus and the defense mechanism of fish, and perhaps lead to the prevention of virus outbreaks in the fish industry.

MATERIALS AND METHODS

Fish and ISKNV infection. Mandarin fish were obtained from fish farms in Nanhai, Guangdong Province, China, and kept in an aquarium with fresh water at 28°C for more than 1 wk before the experiments. Six fish (weight 200.2 ± 38.1 g, body length 24.1 ± 1.2 cm; mean ± SD) were randomly selected from these fish and kept in separate 40 l aquaria. Tank water was passed through a sand-filter with a carbon layer and aerated before use.

To ensure the fish were not infected by ISKNV initially, peripheral blood was collected from the caudal artery and genomic DNA was extracted using the Blood Genomic DNA Purification System (Sino-American Biotechnology, China) according to the manufacturer's instructions. A pair of primers (F1: 5′-CATCAGCAGACCGAGCGTAA-3′, R1: 5′-CCACCTGCTGTTTGCGAGT-3′) were designed according to the VEGF gene of ISKNV and used to amplify the virus gene fragment (596 bp). The final PCR mixture (50 µl per reaction) consisted of 10 mmol l^{-1} Tris-HCl pH 8.5, 1.8 mmol l^{-1} $MgCl_2$, 50 mmol l^{-1} KCl, dNTP mixture (0.2 mmol l^{-1} each), 10 pmol of primers each and an aliquot of the genomic DNA. The PCR conditions were 26 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1 min. At the end of the last cycle, the tubes were further incubated at 72°C for 10 min for the completion of DNA synthesis. The whole blood genomic DNA from fish infected with ISKNV (confirmed by histopathology) was used as a positive control. The PCR products were analyzed on 1% agarose gel. All 6 fish were confirmed ISKNV negative.

Virus preparations were prepared as previously described (He et al. 2002). Briefly, spleens (20 g) of moribund mandarin fish experimentally infected with ISKNV were homogenized with phosphate-buffered saline (PBS), pH 7.2, and centrifuged at 1500 × *g* for 30 min at 4°C. The supernatant was supplemented with 1000 IU ml^{-1} penicillin and 1000 mg ml^{-1} streptomycin and filtered through a 0.22 mm membrane. The experimental fish were infected with ISKNV by intramuscular injection of 1 ml of filtrate. A 50 µl aliquot of the filtrate was inoculated on tryptone soya agar for bacteriological examination. No bacteria were detected after 7 d at 28°C. We injected 3 fish with ISKNV preparations. The other 3 fish were injected with the same volume of PBS (pH 7.2) as controls.

RNA isolation and cDNA synthesis. On the 4th day after injections, all the fish were sacrificed and the spleens removed aseptically for dissection. The spleens from each group of fish were pooled and stored in RNA*later* (Ambion, USA) at –20°C until further use. The anterior kidneys were dissected, genome DNA was extracted and ISKNV infection determined by PCR. Total RNA was extracted with Trizol reagent (Invitrogen, USA) according to the manufacturer's instructions. The integrity of the total RNA was routinely checked by electrophoresis of 20 µg total RNA through 1% agarose, 6.5% formaldehyde gels in 1× MOPS (3-N-morpholino-2-hydroxypropane sulfonic acid) buffer. Complement DNA was synthesized from total RNA using the Super SMART PCR cDNA Synthesis Kit (Clontech, USA). Briefly, 1 µg of total RNA was mixed with 7 µl of 12 µM 3′ SMART CDS Primer IIA and 7 µl of 12 µmol l^{-1} SMART IIA oligonucleotide. The mixture was incubated at 72°C for 2 min. Then, 20 µl of 5× First-strand buffer, 2 µl of 100 mmol l^{-1} DTT, 10 µl of 50× dNTP (10 mmol l^{-1} each), 2.5 µl of RNase inhibitor (40 U $µl^{-1}$) and 5 µl of PowerScript Reverse transcriptase (Clontech, USA) were added to the mixture (total

100 µl) and incubated at 42°C for 90 min. Then, 2.5 µl of the first strand cDNA product was combined with 10 µl of 10× Advantage PCR buffer, 2 µl of 50× dNTP, 2 µl of 5× PCR Primer IIA, 2 µl of 50× Advantage polymerize mix and 81.5 µl of deionized water. The mixture was then amplified by PCR for 18 cycles (94°C for 30 s and 68°C for 6 min each). The PCR product was used for the following SSH experiment.

Suppression subtractive hybridization. SSH was performed with the PCR-select cDNA Subtraction Kit (Clontech, USA). Briefly, Super SMART cDNA from the PBS-injected (Driver) and ISKNV-infected (Tester) fish was digested with restriction endonuclease *Rsa*I to generate short, blunt-ended double-strand cDNAs. After phenol extraction and ethanol precipitation, *Rsa*I-digested tester cDNA was separately ligated with Adaptor 1 and Adaptor 2R at the 5′-end of each strand of the tester cDNA. After denaturation at 95°C for 90 s, the adaptor 1 ligated and adaptor 2R ligated tester cDNA were then separately hybridized with an excess of driver cDNA at 68°C for 8 h. The 2 hybridization samples were mixed together without denaturation and hybridized at 68°C overnight with an excess of fresh denatured driver cDNA. The resulted mixture was diluted to 200 µl then amplified by 2 rounds of PCR to enrich the desired cDNAs containing both adaptors by exponential amplification of these products. The primary PCR was performed with flanking primers against adaptor 1 and adaptor 2R, and the amplified products were used as template in the secondary PCR with nested primers. Finally, the efficiency was evaluated by PCR with primers for the α-actin gene (forward: 5′-AGTCCCCTCCCCCGAAACAC-3′; reverse: 5′-GGGCCCACCAGAGAATAAACCTT-3′) performed on tester (unsaturated) and subtracted cDNA for 20, 25, and 30 cycles. In the reverse subtraction experiment, PBS-injected fish cDNA was used as tester, while ISKNV-injected fish cDNA was used as driver.

Cloning, sequencing and BLAST homology search. The PCR products from the subtraction procedures were cloned into pDrive Cloning Vector (Qiagen, Germany) to create the forward and reverse subtracted cDNA libraries. The libraries were transformed into TG1 competent cells, followed by plating onto agar plates containing ampicillin, X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside), and IPTG (isopropyl-β-D-thiogalactopyranoside). A total of 275 and 111 randomly selected white clones were sequenced, from the forward and reverse libraries respectively. These sequences were edited by using programs to remove vector sequences and ambiguous regions, and then assembled into groups of sequences (clusters) using the SEQMAN subprogram of DNASTAR software (Madison, WI, USA) so as to assemble the longest possible consensus sequences. The consensus sequences of each cluster were used as query sequences to search the non-redundant protein database at the National Center for Biotechnology Information (NCBI) with BLASTx (Benson et al. 2000). For those sequences with no 'hits' found, BLASTn was used to search for similar nucleotide sequences.

Criteria for identification of clones. In those cases for which BLASTx analysis returned E-values < E-5 (indicative of a significant homology), it was common to find significant similarities ('hit') to several sequences in the database. The identifications we assigned were made as follows: if the similarity was markedly higher for one 'hit', that was chosen as the identification of our sequence. In some cases in which E-values were similar for a number of 'hits', we examined the list for molecules from teleost fish and these identifications were assigned. For some sequences that did not meet the criteria of E < E-5, if they showed >90% amino acid identity with known genes or sequences, we assigned the identities to the similar genes.

RT-PCR. RT-PCR was performed to compare the differential expression level of 28 genes in spleen PBS and ISKNV-injected fish. The 18s rRNA gene was used as control. The primers for target genes and 18s rRNA gene are listed in Table 1. Total RNA was prepared from the spleens of fish. 2 µg of total RNA was mixed with 2 µl of 12 µmol ml^{-1} 3′ SMART CDS Primer IIA and 1 µl of random primer (9 mers, 1 mg ml^{-1}). The mixture was incubated at 72°C for 2 min. Then, 5 µl of 5× Moloney murine leukemia virus reverse transcriptase (MMLV) buffer, 0.5 µl of 100 mmol l^{-1} DTT, 2 µl of dNTP (10 mmol l^{-1} each), 0.5 µl of RNase inhibitor (40 U $µl^{-1}$) and 1 Unit of MMLV (Promega, USA) were added to the mixture (total 20 µl) and incubated at 42°C for 90 min for the cDNA synthesis. Equal amounts of cDNAs from the PBS and ISKNV-injected samples were used as template in PCR. The PCR included 28 to 29 cycles of 94°C for 30 s, 52°C for 30 s and extension at 72°C for 40 s. The products were analyzed on 1% agarose gel.

RESULTS

Suppression subtractive hybridization

All the fish injected with ISKNV were confirmed by PCR to be infected by this virus (data not shown). To identify the mRNAs that are up- and down-regulated in ISKNV-infected fish, SSH was performed using spleens of PBS and ISKNV-injected fish. After 2 rounds of hybridization and 2 rounds of PCR to amplify the differentially expressed cDNA, a forward subtraction library and a reverse subtraction library were constructed. We evaluated the subtraction efficiency using the constitutively expressed α-actin gene. After 25

Table 1. Primers used in semi-quantitative RT-PCR used to confirm differences in ISKNV expression levels in mandarin fish *Siniperca chuatsi*

Primer	Sequence (5′–3′)	Product size (bp)
18S-F	ATGGTACTTTAGGCGCCTAC	280
18S-R	TATACGCTATTGGAGCTGG	
IAP-F	CATCGTTTTCATCCCATGTGGACACC	115
IAP-R	TGAGAGGAAGGTTCGGACCGTGCC	
Ubiquitin-activating enzyme E1-F	TTTGAGAAGGATGACGACGGTAACT	242
Ubiquitin-activating enzyme E1-R	AGGTTGATATAAGCTGTGCGGTAAGA	
Interferon-inducible protein Gig 2-F	CACCAGGAAGGCTGCTCAGTCCA	241
Interferon-inducible protein Gig 2-R	CACCGGGCCGTATCTGGAGTCAT	
ISKNV-induced protein 1-F	CTCGGGTCCGGGGATTGTTCCCT	197
ISKNV-induced protein 1-R	TTTCTCCAGACGCTCACTGTCTT	
CD59-F	ACACCTGTGCCAAAATTACCTACCC	143
CD59-R	TTATTTGTTGCAGCCGTCCCAGTTG	
Polyubiquitin-F	ACCCGACGAGACTGTGAGCGACTT	131
Polyubiquitin-R	CTTCGACGTTGTAGTCCGCAAGT	
Ribosomal protein L10F	AAGACCTGCGGAAAGGA	122
Ribosomal protein L10R	GCACCACGCATTCCAGT	
Ribosomal protein L10a-F	GCGGGGCTTTTCATC	141
Ribosomal protein L10a-R	CTCCACCGACTCCACAA	
Ribosomal protein L17-F	GCACATCCAGGTCAACAA	128
Ribosomal protein L17-R	GGACGATCTGCTCCTTCT	
Ribosomal protein L17-2-F	ATCAAGGGGCGTCTCAA	122
Ribosomal protein L17-2-R	CGCTGCCGTATCACCA	
Ribosomal protein L19-F	AGGCTGAGGCTCGTCG	152
Ribosomal protein L19-R	CACTTGTCAGGCAGGATTG	
Ribosomal protein L21-F	TGCTGTCGGCATCATCG	189
Ribosomal protein L21-R	GCAGGCTGGCGTTTCA	
Ribosomal protein L22-F	AAGCAGGTGGTCAAGAAGC	166
Ribosomal protein L22-R	CACCGCCAAGGTTTCC	
Ribosomal protein L30-F	ACGGACTAAACCGCAAAA	141
Ribosomal protein L30-R	CCCCTGACGGATCATCTT	
Ribosomal protein S3a-F	GCCTGTCAGTCCATCTACC	180
Ribosomal protein S3a-R	CTCATAACCATCAGCCCTC	
Ribosomal protein S5-F	GCAACAACGGCAAGAAG	146
Ribosomal protein S5-R	ACCAATACGGGTGGAGTC	
Ribosomal protein S7-F	GACGCCATCTTGGAGGA	175
Ribosomal protein S7-R	CGTCTTTGCCTGTGAGC	
Ribosomal protein S14-F	AAACGTGTTCGGAGTCTGT	120
Ribosomal protein S14-R	TCCGCCTTCACCTTCAT	
Ribosomal protein S19-F	GGAAGTCGCCAGAGGAA	172
Ribosomal protein S19-R	GGCCAGCAATTCTATCCAG	
Ribosomal protein S29-F	GGCTTTCCGACGCTC	157
Ribosomal protein S29-R	GCACTGGCGGCACAT	
Carboxypeptidase A2-F	CCAGTGGCGGCAGCATTGA	131
Carboxypeptidase A2-R	GGTCTCGGAGGCAGTGGGGA	
Mitochondrial ribosomal protein L11-F	AGTGAGGGCAGTGTATGAGA	137
Mitochondrial ribosomal protein L11 -R	GATCGTTGACAACCTTGATG	
Ubiquinol-cytochrome *c* reductase core-F	TGGCAGACACATCCTGAACTACGG	177
Ubiquinol-cytochrome *c* reductase core-R	CGCATTCTGTTGTAGTCGGGTAGC	
Interleukin-8 variant-F	CAACTCCTTTCTACCATCGCCACC	135
Interleukin-8 variant-R	GCTGGGCTTTAGAGATGCAGACCA	
Transmembrane protein 7-F	TGCTACGGAGATGAGGACGACGA	125
Transmembrane protein 7-R	TCATCCTGACAGCAAATGCCCA	
Pleiotropic regulator 1-F	GAGGACGACACTGCCACGGAA	418
Pleiotropic regulator 1-R	GGAGAGTGGCTGATGTTGGTGGAT	
AY909489-unknown protein-F	CAGGAGTCCAGAGGAAACAGCCA	157
AY909489-unknown protein-R	CGTCTAGTCCTTCTGCTGCTCCAA	
AY909493- unknown protein-F	ATTGCCCTGCTGCCCAACATA	140
AY909493-unknown protein-R	GGGAAGTTGCCTTGGCAGTATTCT	

PCR cycles, the α-actin transcript was hardly detectable in the subtracted library compared to that in the unsubtracted sample, suggesting that the SSH procedure successfully suppressed cDNA common to the PBS and ISKNV-injected fish (Fig. 1).

The initial 275 expression sequence tags (ESTs) from the forward SSH library were grouped into 112 consensus sequences, consisting of 49 clusters that contained more than 2 ESTs per cluster and 63 singletons. Thirty-one of the consensus sequences showed significance with known genes (Table 2). They include genes of the ubiquitin–proteasome proteolytic (UPP) pathway (such as those encoding proteasome activator subunit 2, ubiquitin-activating enzyme E1 and polyubiquitin), defence-related genes (such as those encoded ferritin subunits, interferon-inducible protein Gig2, viperin, Mx protein, CC chemokine, hemolytic toxin, interleukin-8 and major histocompatibility class I receptor), an apoptosis-related gene encoding inhibitor of apoptosis protein, a cytoskeletal protein gene (talin) and JFC/EBPb cDNA for CAAT/Enhancer binding protein beta. For one particular sequence, the BLASTx analysis returned no 'hit'. Further BLASTn analysis returned positive hits of JFC/EBPb mRNA for CAAT/Enhancer binding protein beta, with the E-value 2E-72. The similarity was located at the 3′ untranslated region of the target gene. Fragments similar to the polyubiquitin and the interferon-inducible protein Gig 2 were highly abundant in this library, with 11 clones for each.

The 111 ESTs from the reverse library were grouped into 55 consensus sequences, consisting of 17 clusters and 38 singletons. Thirty-two of these consensus sequences showed similarities to other genes (Table 3). They include 17 ribosomal protein genes, 2 cytoskeletal protein genes (tropomyosin4-1 and actinin-associated LIM protein), and those encoded for CD59/neurotoxin/Ly-6-like protein, carboxypeptidase A2, zinc finger protein, testican 3, nascent-polypeptide-associated complex alpha polypeptide, and

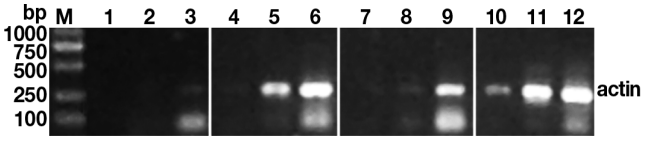

Fig. 1. Evaluation of the subtraction efficiency of subtracted cDNAs. PCRs with α-actin-gene-specific primers were performed on cDNAs subtracted for forward library (lanes 1–3), unsubtracted for forward library (lanes 4–6), subtracted for reverse library (lanes 7–9) and unsubtracted for reverse library (lanes 10–12). PCRs were performed for 20 cycles (lanes 1, 4, 7 & 10), 25 cycles (lanes 2, 5, 8 & 11), and 30 cycles (lanes 3, 6, 9 & 12), respectively. The PCR products were electrophoresed on 1% agarose gel. M: DL-2000 DNA ladder (Shenerg Biocolor, Shanghai, China)

goose-type lysozyme. Fragments similar to the CD59/neurotoxin/Ly-6-like protein were the most abundant in this library, with 7 clones.

The identified sequences were deposited to GenBank. Among these sequences, 6 are first found in fish, including phosphoribosyl pyrophosphate synthetase 1, testican 3, guanine nucleotide binding protein beta subunit 4, solute carrier family 29 (nucleoside transporters) member 4, DnaJ homolog subfamily C member 9 and talin.

From these consensus sequences, the putative complete open reading frames of 2 cDNAs were isolated: CD59/Ly-6/neurotoxin-like protein (GenBank Accession No. AY909432) and 60S ribosomal protein L30 (GenBank Accession No. AY909412).

Confirmation of SSH by RT-PCR

To confirm the result of SSH, different clones were selected for RT-PCR. After normalization with constitutively expressed gene (i.e. 18s rRNA), the expression level of all the 12 genes from the forward library showed an apparent increase in expression in ISKNV-infected fish spleen (Fig. 2). These genes included polyubiquitin, ubiquinol-cytochrome *c* reductase core I protein, interferon-inducible protein Gig2, ubiquitin-activating enzyme E1, transmembrane protein 7, inhibitor of apoptosis protein, pleiotropic regulator 1 and ISKNV-induced protein 1. On the other hand, the expression level of all 16 genes from the reverse library was apparently decreased (Fig. 3). These included CD59/neurotoxin/Ly6, carboxypeptidase A2, and some ribosomal proteins.

DISCUSSION

Although the immune system has evolved mechanisms to eradicate viral-infected cells, many viruses can persist inside the cells to cause latent or chronic

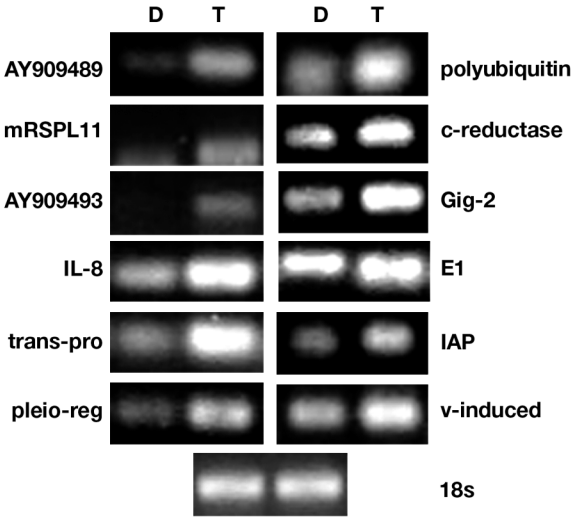

Fig. 2. Comparison of putative up-regulated genes between PBS- and ISKNV-injected fish using RT-PCR. D: PBS-injected fish; T: ISKNV-injected fish; mRSPL11: mitochondrial ribosomal protein L11; c-reductase: ubiquinol–cytochrome c reductase core I protein; Gig-2: interferon-inducible protein Gig2; IL-8: interleukin-8; E1: ubiquitin-activating enzyme E1; trans-pro: similar to transmembrane protein 7; IAP: inhibitor of apoptosis protein; pleio-reg: pleiotropic regulator 1; v-induced: ISKNV-induced protein 1; AY909493: unknown protein (GenBank Access. No. AY909493); AY909489: unknown protein (GenBank Access. No. AY909489)

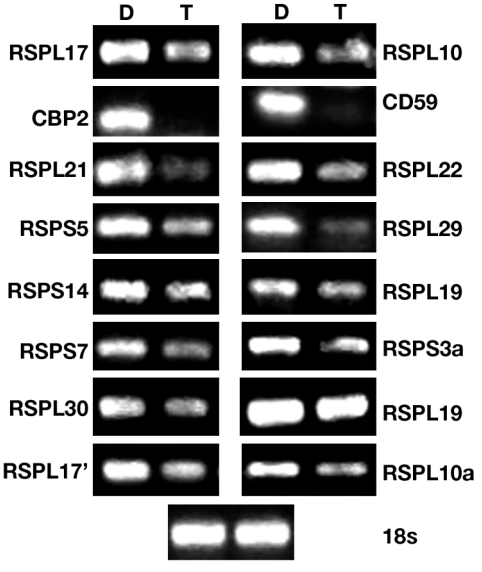

Fig. 3. Comparison of putative down-regulated genes between PBS- and ISKNV-injected fish using RT-PCR. D: PBS-injected fish; T: ISKNV-injected fish; 18s: 18s rRNA; RSPL10: 60s ribosomal protein L10; CBP-2: carboxypeptidase A2; CD59: CD59/Neurotoxin/Ly-6-like gene; RSPL21: 60s ribosomal protein L21; RSPL22: 60s ribosomal protein L21; RSPS5: 40s ribosomal protein S5; RSPS29: 40s ribosomal protein S29; RSPS14: 40s ribosomal protein S14; RSPS19: 40s ribosomal protein S19; RSPS7: 40s ribosomal protein S7; RSPS3a: 40s ribosomal protein S3a; RSPL30: 60S ribosomal protein L30; RSPL19: 60S ribosomal protein L19;. RSPL17′: 60s ribosomal protein L17 (AY909404); RSPL10a: 60s ribosomal protein L10a; RSPL17: 60s ribosomal protein L17 (AY909403)

Table 2. Putative up-regulated genes identified in a cDNA library prepared by SSH using spleens of mandarin fish *Siniperca chuatsi* 4 d after ISKNV-injection. x: number of identical amino acids between query and subject sequences; y: number of amino acids for alignment

Access. no.	Closest database homologue (Access. no.)	Species	Longest fragment (bp) (n)	E Value x/y (% identity)
AY909523	VHSV-induced protein (AAM18479)	*Oncorhynchus mykiss*[a]	421 (3)	2.00E-06 31/71 (43%)
AY909461	Interferon-inducible protein Gig2 (AAP49829)	*Carassius auratus*[a]	637 (11)	3.00E-24 58/85 (68%)
AY395718	Viperin (AY395718)	*Siniperca chuatsi*[a]	475 (3)	3.00E-57 131/131 (100%)
AY392097	Mx protein (AAQ91382)	*Siniperca chuatsi*[a]	429 (4)	2.00E-77 142/142 (100%)
AF327363	Immunoglobulin heavy chain (AF327363)	*Siniperca chuatsi*[a]	303 (1)	4.00E-50 98/98 (100%)
AY909517	CC chemokine (CAC45063)	*Oncorhynchus mykiss*[a]	278 (5)	1.00E-14 35/62 (56%)
AY909475	Interleukin-8 variant 5 (AAN41457)	*Ictalurus punctatus*[a]	406 (1)	6.00E-06 22/78 (28%)
AY909521	Hemolytic toxin (BAC45007)	*Phyllodiscus semoni*	244 (1)	9.00E-04 30/84 (35%)
DQ058109	Ubiquitin-like protein (AAO14689)	*Oncorhynchus mykiss*[a]	229 (11)	7.00E-13 37/63 (58%)
DQ058110	Cytochrome b (BAC99035)	*Siniperca scherzeri*[a]	219 (1)	2.00E-20 51/72 (70%)
AY909514	Phosphoribosyl pyrophosphate synthetase I (XP_420181)	*Gallus gallus*	436 (1)	4.00E-73 140/145 (96%)
AY909449	Ubiquinol-cytochrome c reductase core I protein (AAL74192)	*Oncorhynchus mykiss*[a]	386 (4)	2.00E-47 88/95 (92%)
AY909450	Proteasome activator subunit 2 (AAN40737)	*Paralichthys olivaceus*[a]	395 (1)	2.00E-31 63/75 (84%)
AY909479	Similar to transmembrane protein 7 (XP_236656)	*Rattus norvegicus*	287 (1)	1.00E-06 28/87 (32%)
AY909447	Ubiquitin-activating enzyme E1 (NP_003325)	*Homo sapiens*	264 (4)	6.00E-24 51/86 (59%)
AY909520	FK506 binding protein 5 (CAD87815)	*Danio rerio*[a]	593 (2)	2.00E-26 60/96 (62%)
AY909477	Guanine nucleotide binding protein beta subunit 4 (AAG18442)	*Homo sapiens*	257 (1)	4.00E-39 74/76 (97%)
AY909485	Similar to human opioid growth factor receptor (CAD44463)	*Danio rerio*[a]	232 (1)	2.00E-21 46/68 (67%)
AY909445	Major histocompatibility class I receptor (AAL11413)	*Stizostedion vitreum*[a]	477 (1)	2.00E-67 119/158 (75%)
DQ058111	JFC/EBPb mRNA for CAAT/ Enhancer binding protein beta (AB049813)	*Paralichthys olivaceus*[a]	258 (1)	2.00E-72 177nt/189nt (93%) (BLASTn)
AY909484	Pleiotropic regulator 1 (AAQ91262)	*Danio rerio*[a]	615 (1)	6.00E-10 28/30 (93%)
AY909530	Solute carrier family 29 (nucleoside transporters) member 4 (AAH47592)	*Homo sapiens*	184 (2)	8.00E-07 24/34 (70%)
AY909531	DnaJ homolog, subfamily C, member 9 (NP_598842)	*Mus musculus*	382 (2)	9.00E-37 73/115 (63%)
AY909493	Hypothetical protein FLJ11354 (NP_077024)	*Homo sapiens*	295 (1)	3.00E-05 19/46 (41%)
AY909512	Inhibitor of apoptosis protein (AAP04483)	*Danio rerio*[a]	431 (1)	2.00E-23 48/49 (97%)
AY909429	Similar to mitochondrial riboso mal protein L11 (XP_215146)	*Rattus norvegicus*	168 (1)	7.00E-12 34/55 (61%)
AY909491	Talin (AAF27330)	*Homo sapiens*	271 (1)	2.00E-26 57/59 (96%)
AY909489	Unnamed protein product (CAF91137)	*Tetraodon nigroviridis*[a]	232 (1)	1.00E-16 42/53 (79%)
AY909433	Reverse transcriptase (AAC24982)	Synthetic construct	348 (1)	6.00E-06 20/58 (34%)
AY909435	Reverse transcriptase-like protein (AAD19348)	*Takifugu rubripes*[a]	343 (5)	4.00E-14 36/85 (42%)

[a]Teleost species

infection to the host. The identification of host genes induced by viruses is one of the critical steps in the elucidation of the host defense pathway. To study the interaction between ISKNV and the host mandarin fish, forward and reverse subtractive libraries were constructed from the fish spleen. After ISKNV infection, 31 putative up-regulated genes were identified in the spleen. On the other hand, 32 genes were down-regulated. The major genes identified in this study are described below.

Table 3. Putative down-regulated genes identified in a cDNA library prepared by SSH using spleens of mandarin fish *Siniperca chuatsi* 4 d after ISKNV-injections. x: number of identical amino acids between query and subject sequences; y: number of amino acids for alignment

Access. no.	Closest database homologue (Access. no.)	Species	Longest fragment (bp) (n)	E Value x/y (% identity)
AY738131	Goose-type lysozyme (AY738131)	*Siniperca chuatsi*[a]	133 (1)	6.00E-18 42/44 (95%)
AAQ14862	AAQ14862)	*Siniperca chuatsi*[a]	212 (1)	2.00E-10 28/35 (80%)
AY909463	Ferritin heavy subunit (AAB34575)	*Salmo salar*[a]	387 (4)	3.00E-66 116/128 (90%)
AY909468	Hemoglobin beta-A chain (Q9PVM2)	*Seriola quinqueradiata*[a]	535 (4)	2.00E-69 127/146 (86%)
AY909469	Hemoglobin beta-A chain (Q9PVM2)	*Seriola quinqueradiata*[a]	513 (5)	2.00E-70 127/146 (86%)
AY909470	Hemoglobin alpha chain (P02018)	*Carassius auratus*[a]	196 (2)	8.00E-08 24/29 (82%)
AY909458	Testican 3 (XP_224788)	*Rattus norvegicus*	309 (1)	3.00E-18 38/52 (73%)
AY909432	Neurotoxin/CD59/Ly-6-like protein (AAR20998)	*Ctenopharyngodon idella*[a]	423 (7)	1.00E-09 34/92 (36%)
AY909496	Carboxypeptidase A2 (BAC53787)	*Paralichthys olivaceus*[a]	535 (1)	3.00E-23 49/61 (80%)
AY909538	Nascent-polypeptide-associated complex alpha polypeptide (AAP20156)	*Pagrus major*[a]	102 (1)	0.71 17/17 (100%)
AY909506	Zinc finger (NP_938184)	*Danio rerio*[a]	177 (1)	4.00E-14 35/48 (72%)
AY909403	60S ribosomal protein L17 (AAF61071)	*Paralichthys olivaceus*[a]	441 (1)	6.00E-66 123/137 (89%)
AY909404	60S ribosomal protein L17 (AAC96111)	Dicentrarchus labrax[a]	177 (2)	2.00E-25 58/58 (100%)
AY909401	60S ribosomal protein L10a (AAK95136)	*Ictalurus punctatus*[a]	299 (2)	4.00E-37 74/84 (88%)
AY909424	40S ribosomal protein S17 (NP_001012)	*Homo sapiens*	498 (3)	4.00E-48 95/97 (97%)
AY909417	40S ribosomal protein s3a (O73813)	*Oryzias latipes*[a]	283 (1)	4.00E-28 61/78 (78%)
AY909422	40S ribosomal protein S14 (AAK95196)	*Ictalurus punctatus*[a]	262 (1)	2.00E-33 69/71 (97%)
AY909426	40S ribosomal protein S23 (NP_001016)	*Homo sapiens*	315 (2)	2.00E-45 91/92 (98%)
AY909425	40S ribosomal protein S19 (AAP20214)	*Pagrus major*[a]	271 (1)	9.00E-38 78/81 (96%)
AY909416	40S ribosomal protein S8 (NP_001003)	*Homo sapiens*	483 (1)	2.00E-46 86/94 (91%)
AY909399	60S ribosomal protein L10 (AAK95135)	*Ictalurus punctatus*[a]	194 (1)	9.00E-31 63/64 (98%)
AY909408	60S ribosomal protein L22 (BAB21247)	*Gallus gallus*	281 (1)	1.00E-28 61/65 (93%)
AY909406	60S ribosomal protein L19 (AAH62844)	*Danio rerio*[a]	282 (1)	6.00E-06 23/23 (100%)
AY909418	40S ribosomal protein S5 (AAP20199)	*Pagrus major*[a]	256 (2)	2.00E-42 84/84 (100%)
AY909412	60S ribosomal protein L30 (P58372)	*Ictalurus punctatus*[a]	469 (1)	4.00E-49 95/97 (97%)
AY909428	40S ribosomal protein S29 (AAK95214)	*Ictalurus punctatus*[a]	173 (2)	2.00E-22 45/45 (100%)
AY909419	40S ribosomal protein s7 (P50894)	*Takifugu rubripes*[a]	287 (1)	2.00E-34 72/74 (97%)
AY909407	60S ribosomal protein L21 (AAK95147)	*Ictalurus punctatus*[a]	356 (3)	6.00E-55 101/114 (88%)
AY909515	Tropomyosin4-1(BAC57571)	*Takifugu rubripes*[a]	230 (1)	5.00E-32 70/76 (92%)
AY909526	Actinin-associated LIM protein (CAC33787)	*Salmo salar*[a]	607 (2)	7.00E-83 145/185 (78%)
AY909483	Thoc1 with death domain (AAH54938)	Danio rerio[a]	195 (1)	7.00E-17 42/52 (80%)
AY909532	Unnamed protein (CAF99686)	*Tetraodon nigroviridis*[a]	182 (1)	1.00E-09 34/51 (66%)

[a]Teleost species

Cytokine genes

Among the earliest immune mediators produced upon virus infection, cytokines orchestrate the induction and maintenance of innate and adaptive antiviral responses (Tortorella et al. 2000). Cytokines are powerful antiviral mediators, allowing the clearance of virus infection. After ISKNV infection, the expressions of Mx protein, interferon-inducible protein Gig-2, and viperin (interferon-inducible and antiviral protein) increased. Mx protein is an interferon-induced GTP-binding protein with documented antiviral activity (Leong et al. 1998). The increased expression of these genes suggested that the interferon pathway was stimulated after ISKNV infection. While several interferon cDNAs have been cloned from such fishes as goldfish (GenBank #AY452069), Japanese puffer fish *Takifugu rubripes* (GenBank #AJ583023), spotted green puffer fish *Tetraodon nigrovirides* (Lutfalla et al. 2003), Atlantic salmon (Robertsen et al. 2003), channel catfish (Long et al. 2004) and zebrafish (Altmann et al. 2003), the mandarin fish interferon cDNA has not been cloned. Two inflammatory cytokine genes, CC chemokine and interleukin-8 (CXC chemokine), were found in the forward SSH library. They are the major mediators of the inflammatory response, which indicates that inflammatory response might be essential in the defense of the fish. CD59/Neurotoxin/Ly-6-like protein gene was down-regulated after ISKNV infection. Mammalian CD59 is a complement regulatory protein, which can inhibit complement activation and membrane attack complex (MAC) formation on autologous cells (Fisicaro et al. 2000). Some virus infections decrease the expression levels of host CD59 (Aries et al. 1997, Kawano et al.

1997, Rautemaa et al. 2002). Deficiency or down-regulation of CD59 results in unrestricted autologous MAC formation and, thereby, in higher sensitivity to complement attack. The down-regulation of CD59 in the ISKNV-infected host cells may make these cells more sensitive to complement attack. This may work as an anti-virus mechanism of the host. Further work should be done to confirm this. In the meantime, anaemia was demonstrated to be an important clinical symptom of ISKNV-infected mandarin fish (Weng et al. 1998). The role of CD59/Neurotoxoin/Ly-6-like protein in the formation of anaemia needs to be determined.

Ubiquitin–proteasome pathway (UPP)-related genes

Over the last decade, it has become clear that modification of protein by the covalent attachment of ubiquitin and ubiquitin-related protein is involved in the control of many, if not all, fundamental cellular processes (Coscoy & Ganem 2000). Some viral proteins, as well as being substrates for the UPP, directly target this system for utilization by the virus for its own purposes. First, some viral proteins seem to specifically target major histocompatibility class I molecules for ubiquitination, resulting in the internalization and subsequent degradation of these molecules in the endosomal/lysosomal system (Coscoy & Ganem 2000). Second, in some viruses, ubiquitination of viral capsid proteins may be needed for the maturation and the release of the viral progeny (Strack et al. 2000). In some situations, the virus utilizes the UPP to target a number of important negative cell regulatory proteins for degradation, which is presumably important for the virus. In ISKNV-infected mandarin fish, polyubiquitin, ubiquitin-activating enzyme E1 and proteasome activator subunit 2 genes were up-regulated. This suggested that the ISKNV might recruit the host UPP for its purposes.

Additionally, the ISKNV ORF 99L sequence was found in the forward library. ORF 99L encodes a protein with C3HC4-type zinc finger (RING finger) domain (He et al. 2001). Many RING finger proteins possess ubiquitin ligase E3 activity (Liu 2004). During ISKNV infection, ORF 99L was suggested to be expressed and act as ubiquitin ligase E3, but its specific substrate remains to be identified.

Apoptosis-related gene

Replicating viruses may stimulate suicide of the host cell directly or provoke recognition by cytolytic T cells and NK cells. These immune effector cells induce apoptosis (Tortorella et al. 2000). Apoptosis is a crucial biological process that eliminates virus-infected cells and is considered as an innate response induced to counteract viral infection (Wrzesien-Kus et al. 2004). In many cases, apoptosis has an adverse effect on virus replication. In these situations, viruses may express or induce proteins that can interfere at multiple points in the orderly execution of apoptosis. In this paper, host inhibitor of apoptosis protein (IAP) expression level was increased in spleen cells after ISKNV infection. Mammalian IAPs block apoptosis either by binding and inhibiting caspases or through caspase-independent mechanisms (Wrzesien-Kus et al. 2004). We suggest that the mandarin fish IAP was induced by ISKNV infection and the up-regulation of IAP expression would be advantageous to ISKNV replication. Further work needs to be done to confirm this.

CCAAT/enhancer binding protein beta (C/EBPβ) gene

After ISKNV infection, the C/EBPβ was up-regulated. C/EBPβ are a family of leucine zipper transcription factors comprising 6 members: α, β, δ, ε, γ and ξ, that are critically involved in the regulation of normal cellular differentiation and function in multiple tissues (Lekstrom-Himes & Xanthopoulos 1998). They act as gene regulatory proteins that are either transcriptional repressors or activators (Darlington et al. 1998) and the binding motifs have been identified in regulatory regions of various genes expressed by cells of myelomonocytic lineages, particularly those encoding inflammatory cytokines (IL-6, TNF-α), other cytokines (IL-8 and IL-12) and genes encoding proteins important for macrophage and granulocytic function, including macrophage, granulocyte and granulocyte/macrophage receptor genes (Poli 1998), consequently reflecting the biological significance of C/EBPs as regulators of immune related genes.

C/EBPs bind to virus promoters or proteins and play a role in virus replication. Wang et al. (2003a,b) recently demonstrated that C/EBPα is able to reciprocally transactivate both replication-associated protein and replication and transcription activator promoters of Kaposi's sarcoma-associated herpesvirus (KSHV), and that the introduction of exogenous C/EBPq induces mRNA and protein expression from both of these immediate-early genes in endogenous latent genomes in KSHV-infected PEL cell lines. Furthermore, C/EBPα was reported to bind to the Epstein-Barr virus ZTA protein during induction of the EBV lytic cycle (Wu et al. 2004). C/EBPβ was reported to bind to HIV LTR and repress the transcription of HIV (Nakata 2000). The function of mandarin fish C/EBP will be determined in future research.

Ribosomal protein genes

Iridovirus FV3 infection results in a rapid inhibition of host cell protein synthesis. Translational shut-off appears to be consequence of a complex series of events involving the degradation of host mRNA (Chinchar 2002) but the degraded mRNAs during virus infection were not identified in detail. mRNAs encoding ribosomal proteins in HeLa cells undergo concomitant degradation after infection with herpes simplex virus type 1 (Greco et al. 1997). In this paper, ribosomal protein ESTs L17, L10, L22, L19, L30, L21, S17, S3a, S14, S23, S19, S8, S5, S29 and S7 were identified from the reverse library, which meant the lower mRNA level in ISKNV-infected fish than in mock-infected fish. The ISKNV infection may degrade the mRNA of these genes, inhibiting the synthesis of host cell proteins. Further studies should be done to confirm this.

As SSH can yield false positives, up- or down-regulation of individual gene transcription will require further detailed investigation.

In summary, the down- or up-regulated gene expression profile in the spleen of mandarin fish after ISKNV infection shows that multiple processes are involved in the infection. These results provide insights into individual genes and pathways that constitute the host cell's response to virus infection. Some cDNA sequences responding to virus infection will be of great value in understanding the immune system of the fish and interactions between the fish and ISKNV. Further studies of some of these proteins and processes are currently ongoing.

Acknowledgements. This research was supported by the Chinese National '863' Project (grant no. 2003AA603011), National Natural Science Foundation of China (grant no. 30300266, 30325035) and funds from the Science and Technology Bureau of Guangdong Province.

LITERATURE CITED

Altmann SM, Mellon MT, Distel DL, Kim CH (2003) Molecular and functional analysis of an interferon gene from the zebrafish, *Danio rerio*. J Virol 77:1992–2002

Aries SP, Schaaf B, Hansen F, Weyrich K, Kurowski V, Dennin R, Dalhoff K (1997) Expression of complement receptors and regulatory proteins on alveolar CD4+ lymphocytes from human immunodeficiency virus-1 infected individuals. Eur Respir J 10:1736–1741

Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Rapp BA, Wheeler DL (2000) GenBank. Nucleic Acids Res 28:15–18

Chen XH, Lin KB, Wang XW (2003) Outbreaks of an iridovirus disease in maricultured large yellow croaker, *Larimichthys crocea* (Richardson), in China. J Fish Dis 26: 615–619

Chinchar VG (2002) Ranaviruses (family Iridoviridae): emerging cold-blooded killers. Arch Virol 147:447–470

Chou HY, Hsu CC, Peng TY (1998) Isolation and characterization of a pathogenic iridovirus from cultured Grouper (*Epinephelus* sp.) in Taiwan. Fish Pathol 33:201–206

Chua HC, Ng ML, Woo JJ, Wee JY (1994) Investigation of outbreaks of a novel disease, 'Sleepy Grouper Disease', affecting the brown-spotted grouper, *Epinephelus tauvina* Forskal. J Fish Dis 17:417–427

Coscoy L, Ganem D (2000) Kaposi's sarcoma-associated herpesvirus encodes two proteins that block cell surface display of MHC class I chains by enhancing their endocytosis. Proc Natl Acad Sci USA 97:8051–8056

Danayadol Y, Direkbusarakom S, Boonyaratpalin S, Miyazaki T, Miyata M (1996) An outbreak of iridovirus-like infection in brown-spotted grouper (*Epinephelus malabaracus*) cultured in Thailand. AAHRI (Aquatic Animal Health Research Institute) Newsletter 5:6

Darlington GJ, Ross SE, MacDougald OA (1998) The role of C/EBP genes in adipocyte differentiation. J Biol Chem 273:30057–30060

Diatchenko L, Lau YF, Campbell AP, Chenchik A and 7 others (1996) Suppression subtractive hybridization: a method for generating differentially regulated or tissue-specific cDNA probes and libraries. Proc Natl Acad Sci USA 93: 6025–6030

Fisicaro N, Aminian A, Hinchliffe SJ, Morgan BP, Pearse MJ, D'Apice AJ, Cowan PJ (2000) The pig analogue of CD59 protects transgenic mouse hearts from injury by human complement. Transplantation 70:963–968

Greco A, Laurent AM, Madjar JJ (1997) Repression of beta-actin synthesis and persistence of ribosomal protein synthesis after infection of HeLa cells by herpes simplex virus type 1 infection are under translational control. Mol Gen Genet 256:320–327

He JG, Weng SP, Huang ZJ, Zeng K (1998) Identification of outbreak and infectious diseases pathogen of *Siniperca chuatsi*. Acta Scientiarum Naturalium Universitatis Sunyatseni 5:74–77

He JG, Weng SP, Zeng K, Huang ZJ, Chan SM (2000) Systemic disease caused by an iridovirus-like agent in cultured mandarinfish, *Siniperca chuatsi* (Basilewsky), in China. J Fish Dis 23:219–222

He JG, Deng M, Weng SP, Li Z, Zhou SY, Long QX, Wang XZ, Chan SM (2001) Complete genome analysis of the mandarin fish infectious spleen and kidney necrosis iridovirus. Virology 291:126–139

He JG, Zeng K, Weng SP, Chan SM (2002) Experimental transmission, pathogenicity and physical-chemical properties of infectious spleen and kidney necrosis virus (ISKNV). Aquaculture 204:11–24

Inouye K, Yamano K, Maeno Y, Nakajima K, Matsuoka M, Wada Y, Sorimachi M (1992) Iridovirus infection of cultured red sea bream, *Pagrus major*. Fish Pathol 27:19–27

Jung SJ, Oh MJ (2000) Iridovirus-like infection associated with high mortalities of striped beakperch, *Oplegnathus fasciatus* (Temmink et Schlegel), in southern coastal areas of the Korean peninsula. J Fish Dis 23:223–226

Kawano M, Tsunoda S, Koni I, Mabuchi H, Muramoto H, Yachie A, Seki H (1997) Decreased expression of 20-kD homologous restriction factor (HRF20, CD59) on T lymphocytes in Epstein-Barr virus (EBV)-induced infectious mononucleosis. Clin Exp Immunol 108:260–265

Lekstrom-Himes J, Xanthopoulos KG (1998) Biological role of the CCAAT/enhancer-binding protein family of transcription factors. J Biol Chem 273:28545–28548

Leong JC, Trobridge GD, Kim CH, Johnson M, Simon B (1998) Interferon-inducible Mx proteins in fish. Immunol Rev 166:349–363

Liu YC (2004) Ubiquitin ligases and the immune response. Annu Rev Immunol 22:81–127

Long S, Wilson M, Bengten E, Bryan L, Clem LW, Miller NW, Chinchar VG (2004) Identification of a cDNA encoding channel catfish interferon. Dev Comp Immunol 28:97–111

Lutfalla G, Crollius HR, Stange-Thomann N, Jaillon O, Mogensen K, Monneron D (2003) Comparative genomic analysis reveals independent expansion of a lineage-specific gene family in vertebrates: the class II cytokine receptors and their ligands in mammals and fish. BMC Genomics 4:29. Available at: www.biomedcentral.com/ 1471–2164/4/29

McGrogan DG, Ostland VE, Byrne PJ, Ferguson HW (1998) Systemic disease involving an iridovirus-like agent in cultured tilapia, *Oreochromis niloticus* L.—a case report. J Fish Dis 21:149–152

Nakajima K, Sorimachi M (1995) Production of monoclonal antibodies against red sea bream iridovirus. Fish Pathol 30:47–52

Nakata K (2000) [Tuberculosis and the human immunodeficiency virus infection]. Nihon Hansenbyo Gakkai Zasshi 69:87–92 (in Japanese)

Poli V (1998) The role of C/EBP isoforms in the control of inflammatory and native immunity functions. J Biol Chem 273:29279–29282

Rautemaa R, Helander T, Meri S (2002) Herpes simplex virus 1 infected neuronal and skin cells differ in their susceptibility to complement attack. Immunology 106:404–411

Robertsen B, Bergan V, Rokenes T, Larsen R, Albuquerque A (2003) Atlantic salmon interferon genes: cloning, sequence analysis, expression, and biological activity. J Interferon Cytokine Res 23:601–612

Rodge HD, Kobs M, Macartney A, Frerichs GN (1997) Systemic iridovirus infection in freshwater angelfish, *Pterophyllum scalare* (Lichtenstein). J Fish Dis 20:69–72

Shi CY, Wang YG, Yang SL, Huang J, Wang QY (2004) The first report of an iridovirus-like agent infection in fanned turbot, *Scophthalmus maximus*, in China. Aquaculture 236:11–25

Strack B, Calistri A, Accola MA, Palu G, Gottlinger HG (2000) A role for ubiquitin ligase recruitment in retrovirus release. Proc Natl Acad Sci USA 97:13063–13068

Sudthongkong C, Miyata M, Miyazaki T (2002a) Iridovirus disease in 2 ornamental tropical freshwater fishes: African lampeye and dwarf gourami. Dis Aquat Org 48:163–173

Sudthongkong C, Miyata M, Miyazaki T (2002b) Viral DNA sequences of genes encoding the ATPase and the major capsid protein of tropical iridovirus isolates which are pathogenic to fishes in Japan, South China Sea and Southeast Asian countries. Arch Virol 147:2089–2109

Tortorella D, Gewurz BE, Furman MH, Schust DJ, Ploegh HL (2000) Viral subversion of the immune system. Annu Rev Immunol 18:861–926

Wang SE, Wu FY, Fujimuro M, Zong J, Hayward SD, Hayward GS (2003a) Role of CCAAT/enhancer-binding protein alpha (C/EBPalpha) in activation of the Kaposi's sarcoma-associated herpesvirus (KSHV) lytic-cycle replication-associated protein (RAP) promoter in cooperation with the KSHV replication and transcription activator (RTA) and RAP. J Virol 77:600–623

Wang SE, Wu FY, Yu Y, Hayward GS (2003b) CCAAT/enhancer-binding protein-alpha is induced during the early stages of Kaposi's sarcoma-associated herpesvirus (KSHV) lytic cycle reactivation and together with the KSHV replication and transcription activator (RTA) cooperatively stimulates the viral RTA, MTA, and PAN promoters. J Virol 77:9590–9612

Wang YQ, Lü L, Weng SP, Huang JN, Chan SM, He JG (2006) Molecular epidemiology and phylogenetic analysis of a marine fish infectious spleen and kidney necrosis virus-like (ISKNV-like) virus. Arch Virol (in press) doi: 10.1007/s00705-006-0870-4

Weng SP, He JG, Zeng K, Huang ZJ (1998) Infectious spleen and kidney necrosis virus infection in *Siniperca chuatsi* (Basilersky)—histopathology and relationship with HB, RBC, and WBC. South China Normal University, Natural Science 82:71–89

Weng SP, Wang YQ, He JG, Deng M, Lu L, Guan HJ, Liu YJ, Chan SM (2002) Outbreaks of an iridovirus in red drum, *Sciaenops ocellata* (L.), cultured in southern China. J Fish Dis 25:681–685

Williams T (1996) The iridoviruses. Adv Virus Res 46:345–412

Wrzesien-Kus A, Smolewski P, Sobczak-Pluta A, Wierzbowska A, Robak T (2004) The inhibitor of apoptosis protein family and its antagonists in acute leukemias. Apoptosis 9:705–715

Wu FY, Wang SE, Chen H, Wang L, Hayward SD, Hayward GS (2004) CCAAT/enhancer binding protein alpha binds to the Epstein-Barr virus (EBV) ZTA protein through oligomeric interactions and contributes to cooperative transcriptional activation of the ZTA promoter through direct binding to the ZII and ZIIIB motifs during induction of the EBV lytic cycle. J Virol 78:4847–4865

Editorial responsibility: Jo-Ann Leong, Kaneohe, Hawaii, USA

Submitted: October 1, 2005; Accepted: July 17, 2006
Proofs received from author(s): November 20, 2006