Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Interleukin-17 suppresses grass carp reovirus infection in *Ctenopharyngodon idella* kidney cells by activating NF-κB signaling

Yunshan Zhang\(^a,1\), Xing Zhang\(^b,1\), Zi Liang\(^a,1\), Kun Dai\(^a\), Min Zhu\(^a\), Mingtian Zhang\(^a\), Jun Pan\(^a\), Renyu Xue\(^a,b\), Guangli Cao\(^a,b\), Jian Tang\(^a\), Xuehong Song\(^a\), Xiaolong Hu\(^a,b\), Chengliang Gong\(^a,b,⁎\)

\(^a\) School of Biology and Basic Medical Sciences, Soochow University, Suzhou, Jiangsu 215123, China
\(^b\) Agricultural Biotechnology Research Institute, Agricultural biotechnology and Ecological Research Institute, Soochow University, Suzhou 215123, China

**A R T I C L E  I N F O**

**Keywords:** Interleukin-17
GrCV
CIK cells
Virus infection
NF-κB

**A B S T R A C T**

The grass carp accounts for a large proportion of aquacultural production in China, but the hemorrhagic disease caused by grass carp reovirus (GrCV) infection often causes huge economic losses to the industry. Interleukin 17 (IL-17) is an important cytokine that plays a critical role in the inflammatory and immune responses. Although IL-17 family members have been extensively studied in mammals, our knowledge of the activity of IL-17 proteins in teleosts in response to viral infection is still limited. In this study, the role of IL-17 in GrCV infection and its mechanism were investigated. The expression levels of IL-17AF1, IL-17AF2, and IL-17AF3 in *Ctenopharyngodon idella* kidney (CIK) cells gradually increased from 6 h after infection with GCV. The nuclear translocation of p65, which acts in the NF-κB signaling pathway, was also increased by GCV infection. The overexpression of IL-17AF1, IL-17AF2, or IL-17AF3 also promoted the nuclear translocation of p65 and the levels of phospho-IκBα in CIK cells, and reduced the expression of the viral structural protein VP7. An NF-κB signal inhibitor abolished the inhibition of GCV infection by IL-17 proteins. These results suggested that the NF-κB signaling pathway was activated by the overexpression of IL-17 proteins, resulting in the inhibition of viral infection. In conclusion, in this study, we demonstrated that IL-17AF1, IL-17AF2, and IL-17AF3 acted as immune cytokines, exerting an antiviral effect by activating the NF-κB signaling pathway.

1. Introduction

Cytokines are pleiotropic molecules that play important roles in many biological processes, including growth, development, differentiation, immunity, inflammation, and hematopoiesis (Jin et al., 2008; Li et al., 2015). Interleukins (ILs) are a group of cytokines that were first detected in leukocytes, and regulate the immune response. The interleukin 17 (IL-17) family has been comprehensively investigated in mammals, and comprises six members (IL-17A–IL-17F) with different bioactivities. IL-17 s are produced by a unique T-cell subset, the T-helper 17 (Th17) cells, and a variety of innate immune cells (Korn et al., 2009). IL-17 s recruit neutrophils and induce a variety of cells to release inflammatory factors, which participate in a variety of biological processes (Murugaiyan et al., 2008). In addition to the mammalian genes, homologous genes have been found in other vertebrates and invertebrates (Kono et al., 2011; Wu et al., 2013). Because of their strong genetic similarity to mammalian IL-17, it has been suggested that the roles of the IL-17 proteins are conserved among the vertebrates (Min and Lillehoj, 2002).

Although the mammalian IL-17 family members have been studied extensively, our knowledge of the IL-17 s in teleosts is still limited. The il17 family genes in fish (which encode IL-17A/F1–3, IL-17C, and IL-17D) were first cloned from zebrafish (*Danio rerio*), and share 20%–50% identity with the corresponding IL17 genes in humans (Gunimaladevi et al., 2006). The il17 family genes were subsequently identified in other fish, including the Japanese pufferfish (*Takifugu rubripes*) (Korenaga et al., 2010), Atlantic salmon (*Salmo salar*) (Kumari et al., 2009; Wang et al., 2015), rainbow trout (*Oncorhynchus mykiss*) (Wang et al., 2010; Monte et al., 2013), grass carp (*Ctenopharyngodon idella*) (Du et al., 2014), and catfish (*Ictalurus punctatus*) (Wang et al., 2014) and in the jawless lamprey (*Lethenteron japonicum*) (Tsutsui et al., 2007). These il17 genes showed different constitutive expression patterns in the tissues of fish species, suggesting that the IL-17 proteins have various complex functions in different tissues (Du et al., 2014). At

---

*Corresponding author at: School of Biology and Basic Medical Sciences, Soochow University, No.199 Ren’ai Road, Dushu Lake Higher Education Town, Suzhou Industrial Park, Suzhou, 215123, China.

E-mail addresses: xh2013@suda.edu.cn (X. Hu), gongcl@suda.edu.cn (C. Gong).

† These authors contributed equally to this work.

https://doi.org/10.1016/j.aquaculture.2020.734969

Received 23 September 2019; Received in revised form 21 December 2019; Accepted 15 January 2020

Available online 16 January 2020

0044-8486/ © 2020 Elsevier B.V. All rights reserved.
present, the response of IL-17 proteins to the infection of grass carp reovirus (GCRV) is still unclear and there is still largely unknown about the mechanisms underlying GCRV infection. GCRV causes grass carp hemorrhagic disease with high mortality rates, and in consequence, brought huge economic losses to the grass carp aquaculture industry.

In this study, we evaluated the effects of grass carp (C. idella) IL-17AF1, IL-17AF2, and IL-17AF3 on GCRV infection and studied the molecular mechanism by which they exert their antiviral effects. We found that all three proteins acted as immune cytokines, exerting their antiviral effect by activating the NF-κB signaling pathway. These findings not only define the roles of the IL-17 family members in response to viral challenge, but also provide the basis for characterizing the il17 family genes in teleosts.

2. Materials and methods

2.1. Cells and virus

Ctenopharyngodon idellus kidney (CIK) cells were cultured in Medium 199 (Gibco, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum (FBS) at 28 °C. GCRV-873 strain was kindly gifted by Professor Hui Chen (Jiangsu Center for Control and Prevention of Aquatic Animal Infectious Disease, Nanjing, China).

2.2. Antibodies and pharmaceuticals

The primary antibodies used in this study included mouse polyclonal antibodies directed against IL-17AF1, IL-17AF2 and IL-17AF3, provided by Professor Xuehong Song (Soochow University, Suzhou, Jiangsu, China). The anti-NF-κB (p65) (10745-1-AP), anti-lamin B (12987-1-AP), and anti-α-tubulin (11224-1-AP) antibodies were purchased from the Proteintech Group (Wuhan, Hubei, China). Anti-phospho (p)-IKB-α (CS-2859) was purchased from Cell Signaling Technology Company. A mouse polyclonal antibody directed against the viral structural protein VP7 of GCRV was prepared in our laboratory (Liu et al., 2016). Horseradish peroxidase (HRP)-conjugated goat anti-rabbit immunoglobulin G (IgG; 10285-1-A) and anti-mouse IgG (10283-1-AP) antibodies, used as the secondary antibodies, were purchased from the Proteintech Group.

2.3. Multiple sequence alignment and structural domain analysis

Based on the coding sequences of C. idella IL-17AF1 (KC978892.1), IL-17AF2 (KP41231.1), and IL-17AF3 (KP412313.1) mRNAs, the amino acid sequences were extracted from the protein database with the corresponding accession numbers (AGT55826.1, AKM20921, and AKM20919, respectively). Other IL-17 genes were extracted from National Center for Biotechnology Information (NCBI) Batch Entrez (https://www.ncbi.nlm.nih.gov/sites/batchentrez?) (Supplementary Table 1). A multiple sequence alignment of IL-17AF1, IL-17AF2, and IL-17AF3 proteins was constructed with the Cluster W software. The key structural features in the proteins from different species were analyzed with the new ENDscript server (Robert and Gouet, 2014). The structural domains in these IL-17 proteins were analyzed with the Multiple Em for Motif Elicitation (http://meme-suite.org/meme_5.0.4/) (Bailey and Elkan, 1994).

2.4. CIK cells challenged with GCRV

CIK cells (1 × 10^5 cells) were seeded in 6-well plates and cultured to the exponential phase. Viral strain GCRV-873 was used to infect the cells (multiplicity of infection [MOI] = 5). After incubation at 4 °C for 30 min, the cell supernatant was replaced with complete medium and culture continued. Normal CIK cells (without GCRV infection) were used as the control group. These experiments were replicated with three times.
2.5. Total protein extraction and SDS-polyacrylamide gel electrophoresis

At 6, 12, and 24 h postinfection (hpi), the total proteins were extracted from the GCRV-infected and normal control CIK cells with the Total Protein Extraction Kit (BestBio, Shanghai, China), according to the manufacturer's instructions. The quality and quantity of the extracted proteins were evaluated with the Bradford Kit (500–0001, Bio-Rad), according to the manufacturer's instructions. Total proteins (20 μg) from the different samples were boiled with 4× SDS loading buffer and resolved with 12% SDS-PAGE.

Fig. 2. Conserved sites and domains of the IL-17 proteins, predicted with Multiple Em for Motif Elicitation. GenBank accession numbers for IL-17 are listed in Supplementary Table 1.

Fig. 3. Expression patterns of IL-17AF1, IL-17AF2, and IL-17AF3 at different times after challenge with GCRV. Asterisks (***, **, and *) indicate significant differences (p < .001, p < .01, and p < .05, respectively) between the challenged and control CIK cells.
Cells (1 × 10^5) were infected with GCRV (MOI = 5) and incubated for 6, 12, or 24 h. The nuclear proteins were extracted with specific primary antibodies, anti-α-tubulin antibody, or anti-lamin B (a nuclear marker) antibody overnight at 4 °C. The membranes were then incubated with HRP-conjugated goat anti-rabbit or anti-mouse IgG antibody for 1 h at room temperature, and developed after the proteins were separated with SDS-PAGE, they were incubated with specific primary antibodies, anti-α-tubulin antibody, or anti-lamin B (a nuclear marker) antibody overnight at 4 °C. The membranes were then incubated with HRP-conjugated goat anti-rabbit or anti-mouse IgG antibody for 1 h at room temperature, and developed with Super ECL Western Blotting Substrate (Coolarber, Beijing, China).

2.7. NF-κB activation detection

Cells (1 × 10^5) were infected with GCRV (MOI = 5) and incubated for 6, 12, or 24 h. The nuclear proteins were extracted with the Nucleoprotein Extraction Kit (Beyotime, Shanghai, China), according to the manufacturer’s protocol. Briefly, GCRV-infected CIK cells were lysed with RIPA buffer (Beyotime) for 30 min on ice. The lysed samples were collected and centrifuged at 12,000 × g (4 °C) for 30 min and the supernatants were discarded. The precipitate was treated with 100 μl of buffer C and centrifuged at 12,000 × g (4 °C) for 30 min. The supernatant was transferred into a fresh tube for analysis with western blotting. The p65 subunit is a major component of the NF-κB complex that is responsible for its transactivation, and was detected with an anti-NF-κB (p65) antibody. An anti-lamin B antibody was used to detect the nuclear marker. These experiments were replicated with three times.

2.8. Plasmid construction, transfection and infection

The coding sequences of *C. idella* IL-17AF1 (KC978892.1), IL-17AF2 (KP412312.1), and IL-17AF3 (KP412313.1) mRNAs were inserted into the HindIII and XhoI sites of the eukaryotic expression vector pcDNA3.1 (Invitrogen, USA) to generate plasmids pcDNA3.1–IL-17AF1, pcDNA3.1–IL-17AF2, and pcDNA3.1–IL-17AF3, respectively. CIK cells (10^5 cells/3 cm dish) were transfected separately with each of the recombinant plasmids (2 μg) with X-tremeGENE HP DNA Transfection Reagent (Roche, Germany) and the cells were allowed to grow to approximately 70%-85% confluence, according to the manufacturer’s protocol. An identical batch of cells was transfected with the empty pcDNA3.1 vector as the control. The CIK cells overexpressing IL-17AF, IL-17AF2 or IL-17AF3 were infected with GCRV (MOI = 5). After 24 h, the CIK cells were harvested to detect the expression of VP7 by GCRV with western blotting. All transfections were carried out in triplicate.

2.9. Cell viability

CIK cells were cultured for 12 h in 96-well plates before they were treated with different concentrations of BAY 11–7082 for 24 h. The cell viability was then estimated with a 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay (Xue et al., 2015). Absorbance was detected at a wavelength of 490 nm, with a reference wavelength of 595 nm.

2.10. Inhibitor treatment

CIK cells (10^5 cells/3 cm dish) were transfected with 2 μg of pcDNA3.1–IL-17AF1, pcDNA3.1–IL-17AF2, or pcDNA3.1–IL-17AF3. At 48 h post-transfection, the CIK cells were pretreated with DMSO (final concentration, 10 μM) either alone or with the NF-κB inhibitor BAY11–7082 (final concentration, 10 μM) for 24 h, washed three times with PBS, and then infected with GCRV (MOI = 5) without inhibitor. After 24 h, the CIK cells were harvested to detect the expression of VP7 by GCRV with western blotting. These experiments were carried out in triplicate.

2.11. ImageJ-based quantitative analysis of western blotting data

A quantitative analysis of the visible bands on the western-blotted membrane was performed with the ImageJ software. The integrated density values for the bands of the detected protein were normalized to that of α-tubulin or lamin B. The statistical significance of the quantified western blot data was calculated based on three independent experiments. The original data for western blotting was shown in the Supplementary materials.

3. Results

3.1. Sequence characterization of IL-17AF1, IL-17AF2, and IL-17AF3

To analyze the sequences of *C. idella* IL-17AF1, IL-17AF2, and IL-17AF3, the protein sequences of IL-17A, IL-17F, IL-17AF1, IL-17AF2, and IL-17AF3 from mammals and other species were extracted from the NCBI protein database. Analysis of these amino acid sequences indicated that IL-17AF1, IL-17AF2, and IL-17AF3 contain putative signal peptides of 27, 23, and 21 amino acids, respectively. A multiple sequence alignment of these IL-17 family proteins showed that the *C. idella* IL-17 proteins contain the region that characterizes the IL-17 superfamily, which was located in the C-terminal region and includes four cysteine residues (Fig. 1). The domain motifs of these IL-17 proteins were analyzed and the results showed that IL-17AF1, IL-17AF2, and IL-17AF3 contained the domains conserved in the IL-17 family proteins of other vertebrates and other species. The order of all of the domains in the grass carp proteins was similar to that in other species, whereas a homology analysis of IL-17AF1, IL-17AF2, and IL-17AF3 showed that the three proteins share low homology with each other (Fig. 2). These data suggested that IL-17AF1, IL-17AF2, and IL-17AF3 had roles similar to those of the corresponding proteins identified in other vertebrate species.

3.2. IL-17 proteins involved in antiviral immunity

To explore the response of the IL-17 proteins to GCRV infection, GCRV-infected CIK cells were collected and analyzed with western blotting, using antibodies directed against the three IL-17 proteins. The expression profiles of IL-17AF1, IL-17AF2, and IL-17AF3 over time were
Fig. 5. IL-17 proteins activated the NF-κB signaling pathway to inhibit GCRV infection. (A) Effects of the overexpression of IL-17AF1 on the nuclear translocation of NF-κB (p65), the phosphorylation of IκB protein, and VP7 expression. (B) Effects of IL-17AF2 overexpression on NF-κB (p65) nuclear translocation, IκB protein phosphorylation, and VP7 expression. (C) Effects of IL-17AF3 overexpression on NF-κB (p65) nuclear translocation, IκB protein phosphorylation, and VP7 expression. Asterisks (⁎ and ⋆) indicate significant differences (p < .01 and p < .05).
analyzed in CIK cells challenged with GCRV. The expression levels of IL-17AF1, IL-17AF2, and IL-17AF3 gradually increased from 6 hpi in the GCRV-infected CIK cells (Fig. 3). Our results indicated that the expression of IL-17AF1, IL-17AF2, and IL-17AF3 was responsive to GCRV infection, suggesting that the IL-17 proteins were involved in the immune response to viral challenge.

3.3. NF-κB (p65) nuclear translocation is promoted by GCRV infection

The conserved NF-κB signaling pathway is associated with a variety of physiological and pathological processes (Ma and Hottinger, 2016). In mammals, there are five transcription factors in the NF-κB family (P65/RELA, RELB, CREL, P50, and P52) with domains for dimerization, nuclear localization, and DNA binding (O'Dea and Hoffmann, 2009). In unstimulated cells, NF-κB dimers, the inactive form, are mainly retained in the cytoplasm, whereas with stimulation, including by cytokines, microbial products, and stress, canonical NF-κB signaling is activated when the IκB kinase (IKK) complex phosphorylates the IκB proteins, resulting in the translocation of cytoplasmic NF-κB to the nucleus to regulate the expression of its target genes (Thompson et al., 1995; Kingeter et al., 2010).

To understand the effects of GCRV infection on the NF-κB signaling pathway, the NF-κB (p65) protein in the nuclei of CIK cells was detected with western blotting. Our results showed that the nuclear translocation of the NF-κB (p65) protein was induced by GCRV infection. These results indicated that the NF-κB signaling pathway was activated by GCRV infection, because GCRV promoted p65 nuclear translocation (Fig. 4).

3.4. IL-17 proteins inhibit GCRV infection via the NF-κB signaling pathway

To understand the relationship between the IL-17 family and the NF-κB signaling pathway, the IL-17 proteins of C. idellus (IL-17AF1, IL-17AF2, and IL-17AF3) were overexpressed in CIK cells and the activation of the NF-κB signaling pathway was detected. NF-κB is known to be activated by the phosphorylation of IκB-α (Baeuerle et al., 1996). The nuclear translocation of NF-κB (p65) and the cytoplasmic translocation of p-IκB-α were significantly increased by the overexpression of the IL-17 proteins (Fig. 5A, B, and C). NF-κB (p65) was significantly translocated to the nucleus when the IL-17 proteins were expressed from the vectors, indicating that these proteins activate the NF-κB signaling pathway. When we evaluated the effects of IL-17 protein expression on GCRV infection, the expression of VP7 in the GCRV-infected cells was significantly suppressed by the overexpression of IL-17AF1, IL-17AF2, or IL-17AF3 (Fig. 5A, B, and C). These results indicated that the IL-17 proteins activated the NF-κB signaling pathway by promoting the nuclear translocation of NF-κB (p65) and the phosphorylation of IκB-α, thus suppressing GCRV infection.

3.5. Bay 11–7082 inhibitor eliminated the inhibition of GCRV infection by IL-17 proteins

Bay 11–7082, a irreversible inhibitor of tumor necrosis factor α (TNF-α)-induced IκB-α phosphorylation, inactivates the NF-κB signaling pathway, so it was selected to treat CIK cells overexpressing IL-17AF1, IL-17AF2, or IL-17AF3. The cytotoxicity of Bay 11–7082 and the different concentrations (2.5, 5, 10, or 20 μM) required to inhibit CIK cells were estimated with an MTT assay. The CIK cells tolerated up to 10 μM Bay 11–7082 (Fig. 6). Therefore, 10 μM Bay 11–7082 was used to treat the CIK cells. The overexpression of IL-17AF1, IL-17AF2, or IL-17AF3 in the CIK cells significantly promoted the nuclear translocation of NF-κB (p65) and suppressed GCRV infection. The expression of GCRV VP7 increased when the NF-κB signaling pathway was inactivated with the NF-κB inhibitor Bay 11–7082 (Fig. 7A, B, and C). These results indicated that the IL-17 proteins inhibited GCRV infection by activating the NF-κB signaling pathway.

4. Discussion

The grass carp accounts for a large proportion of aquacultural production in China, but the hemorrhagic disease caused by GCRV often brought huge economic losses in the grass carp aquaculture industry. However, because of its large with a long reproductive cycle, it is hard to study the antiviral mechanisms in the grass carp in vivo (Lin et al., 2019). CIK cells can be infected by GCRV, and this infection is characterized by the systematic induction of a mild host immune response, involving a variety of classical antiviral signal transduction pathways (Ji et al., 2018; Yu et al., 2017; He et al., 2017). In our previous study, CIK cells were used to study the RNA expression patterns in response to viral infection, including circRNAs, mRNAs, and miRNAs. Our results showed that numerous key immune signaling pathways related to the differentially expressed RNAs were enriched and we predicted that they played crucial roles in the C. idellus response to viral infection (Liu et al., 2019). CIK cells can also be used to investigate the functions of proteins in vitro. Therefore, in the present study, CIK cells were selected as the model in which to study the antiviral mechanism of the IL-17 proteins in response to GCRV infection. The IL-17 cDNAs and their cognate proteins share features with their orthologues in mammals and other species. A multiple sequence alignment including these orthologues showed that the C. idellus proteins had similar structural characteristics and the four conserved cysteine residues that characterize IL-17.

Great progress has recently been made in understanding antiviral immunity in teleosts. However, studies of the regulatory mechanisms of the antiviral signaling pathways in the grass carp are far behind the corresponding studies in mammals and other species (Rao and Su, 2015). Like mammals, the lower vertebrates, including fish, are armed with both adaptive and innate immunity, and the innate immune response plays crucial role in defending the fish against invading pathogens (Aoki et al., 2013; Nagasawa et al., 2014). However, at present, the response of the IL-17 family proteins to viral infection and the antiviral mechanisms of teleosts are unclear. The IL-17A and IL-17F proteins belong to the IL-17 family, and are secreted by Th17 and other immune cells (Kondo et al., 2009; Sutton et al., 2009). Normally, IL-17A and IL-17F are secreted as homodimers or heterodimers. They act as proinflammatory cytokines to induce cytokines and chemokines expression, which play crucial roles in the innate and adaptive immune responses (Du et al., 2015; Iwakura et al., 2011; Kolls and Linden, 2004). The sequences of IL-17A/F1, IL-17A/F2, IL-17A/F3, and other members of the IL-17 family were characterized (Du et al., 2014; Du et al., 2015), but the bioactivities of grass carp IL-17A/F1, IL-17A/F2, and IL-17A/F3 during GCRV infection are still unknown. An analysis of the expression patterns of the IL-17 family members in teleost tissues

---

**Fig. 6.** CIK cells were treated with Bay 11–7082 at the indicated concentrations. Cell viability was assessed with an MTT assay.

---

**Fig. 7.** VP7 expression in CIK cells treated with different concentrations (2.5, 5, 10, or 20 μM) of Bay 11–7082. (A) VP7 expression in CIK cells overexpressing IL-17AF1, IL-17AF2, or IL-17AF3. (B) VP7 expression in CIK cells treated with Bay 11–7082 and the overexpression of IL-17AF1, IL-17AF2, or IL-17AF3.

---

**Fig. 8.** The expression of GCRV VP7 in CIK cells treated with different concentrations (2.5, 5, 10, or 20 μM) of Bay 11–7082. (A) VP7 expression in CIK cells overexpressing IL-17AF1, IL-17AF2, or IL-17AF3. (B) VP7 expression in CIK cells treated with Bay 11–7082 and the overexpression of IL-17AF1, IL-17AF2, or IL-17AF3.
Fig. 7. Inhibitor Bay 11–7082 eliminated the inhibition of GCRV infection by overexpressing of IL-17 proteins in CIK cells. (A) Effects of Bay 11–7082 inhibitor on GCRV infection and NF-κB (p65) nuclear translocation in IL-17AF1-overexpressing CIK cells. (B) Effects of Bay 11–7082 inhibitor on GCRV infection and NF-κB (p65) nuclear translocation in IL-17AF2-overexpressing CIK cells. (C) Effects of Bay 11–7082 on GCRV infection and NF-κB (p65) nuclear translocation in IL-17AF3-overexpressing CIK cells. Asterisks (⁎⁎) indicate significant difference (p < .01) and # indicates no significant difference (p > .05).
showed that they are present in several crucial immune tissues (Hansen and Zapata, 1998). IL17AF1, which is most strongly expressed, is expressed in the grass carp thymus, spleen, gill, and skin (Du et al., 2015). The expression of the trout il17af2 gene was relatively high in the intestine and gill (Monte et al., 2013). In zebrafish, IL17AF1 protein expression was only detected in the intestine (Gunimaladevi et al., 2006). In fugu, il17af1 is not expressed in the intestine, head kidney, thymus, spleen, gill, or skin (Korenaga et al., 2010). These results showed that the IL-17 family members showed different patterns of tissue-specific expression, which suggested that they played distinct roles in the immune activities of fish (Korenaga et al., 2010; Lubberts et al., 2005). These findings prompted us to explore the roles of the IL-17 family members during viral infection.

The results of GCRV challenge showed that the expression levels of IL-17AF1, IL17AF2, and IL17AF3 were time-dependently upregulated after GCRV infection. When stimulated with lipopolysaccharide (LPS) or Aeromonas hydrophila subsp. hydrophila infection, the expression of the IL-17 proteins was induced, suggesting that the fish IL-17 proteins not only shared expression profiles after inflammatory stimulation (Kumari et al., 2009; Wang et al., 2010; Monte et al., 2013; Tsutsui et al., 2007; Zhang et al., 2013), but also participated in the host’s defenses against bacterial infection (Du et al., 2015). However, our findings indicated that the expression of the IL-17 proteins was induced by GCRV infection with time course, suggesting that the IL-17 proteins participated in the host’s defense against viral infection.

To test our hypothesis that IL-17 proteins played important roles in the antiviral activity of C. idellus and to identify the signal transduction pathway involved, the effect of GCRV infection on the NF-κB signaling pathway was investigated in CIK cells. Like other species, fish have evolutionarily and functionally conserved pattern recognition receptors (PRRs), which are mainly responsible for sensing pathogen-associated molecular patterns (Aoki et al., 2013; Takeuchi and Akira, 2010). Upon viral challenge, PRRs activate the antiviral signaling pathways by transmitting signals to downstream adaptor molecules (Goubau et al., 2013; Palgi, 2011; Verrier et al., 2011). Recent reports have shown that the canonical NF-κB-dependent signaling pathway not only responds to bacterial and fungal infections, but is also involved in antiviral activity (Costa et al., 2009; Zambon et al., 2005; Liu et al., 2017). During viral stimulation, canonical NF-κB signaling is activated when the IκB kinase (IKK) complex phosphorylates the IκB proteins, causing cytoplasmic NF-κB (p65) translocate to the cell nucleus to regulate its target genes (Thompson et al., 1995; Kinger et al., 2010). From our findings, the nuclear translocation of NF-κB (p65) was promoted by GCRV infection, suggesting that the NF-κB signaling pathway was activated by the virus. NF-κB (p65) nuclear translocation and the expression of p-IκB-α were also induced by the overexpression of IL-17AF1, IL17AF2, or IL17AF3 in CIK cells, indicating that these IL-17 proteins activated the NF-κB signaling pathway with unknown mechanism. We speculated that IL-17 production inhibits GCRV infection via the activation of NF-κB signaling pathway. Our findings on the antiviral regulatory mechanism of IL-17 are similar to those reported for varicella-zoster virus and SARS coronavirus (Doshch et al., 2009; Wang et al., 2005).

Although NF-κB is considered a key transcription factor for a variety of antiviral cytokine genes (Hayden and Ghosh, 2008), some viruses (including spring viremia of carp virus and influenza virus) use the canonical NF-κB signaling pathway for replication (Liu et al., 2017; Wurzer et al., 2004; Chen et al., 2012; Lee and Kleibokker, 2005; Nimmerjahn et al., 2004), whereas other viruses (respiratory syncytial virus and Rotavirus) use the noncanonical NF-κB signaling pathway for infection (Moral-Hernandez et al., 2018; Parkishit Bagchi et al., 2013).

In this study, GCRV infection was suppressed by the overexpression of IL-17AF1, IL17AF2, or IL17AF3 in CIK cells, suggesting that GCRV infection upregulated IL-17 protein expression, which further promoted the nuclear translocation of NF-κB (p65) to regulate the expression of antiviral genes. To further understand how IL-17 proteins directly induce the NF-κB signaling pathway, Bay 11–7082, a irreversible inhibitor of TNF-α-induced IκB-α phosphorylation, was used to inactivate the NF-κB signaling pathway. We then investigated the effect of IL-17 overexpression on GCRV infection. This inhibitor of the NF-κB signaling pathway abolished the inhibition of GCRV infection caused by the overexpression of IL-17 proteins in CIK cells.

In summary, the results of this study demonstrated that the IL-17 family proteins acted as immune cytokines, exerting an antiviral effect by activating the NF-κB signaling pathway. Our data not only defined the roles of the IL-17 family proteins in response to viral challenge, but also provided the basis for the characterization of the IL17 family genes in teleosts.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This study was supported financially by the Key Research and Development Program of Jiangsu Province (Modern Agriculture) (BE2016322), the Triple-New Project of Aquaculture of Jiangsu Province of China (D2017-3), the China Postdoctoral Science Foundation (2019M651952), the National Natural Sciences of China (31777896), and a project funded by the Priority Academic Program of Development of Jiangsu Higher Education.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2020.734969.

References

Aoki, T., Hikima, J., Hwang, S.D., Jung, T.S., 2013. Innate immunity of finfish: prirmordial conservation, 2nd function of viral RNA sensors in teleosts. Fish Shellfish Immunol. 35 (6), 1689-1702.
Banerjee, P.A., Baltimore, D., NF-kappa, B., 1996. Ten years after. Cell 87 (1), 13–20.
Bailey, T.L., Elkan, C., 1994. Fitting a mixture model by expectation maximization to discover motifs in biopolymers. Proc. Int. Conf. Intell. Syst. Mol. Biol. 2, 28–36.
Chen, X., Ren, F., Hesketh, J., Shi, X., Li, J., Gan, F., Huang, K., 2012. Reactive oxygen species regulate the replication of porcine circovirus type 2 via NF-kappaB pathway. Virology 426 (1), 66–72.
Costa, A., Jan, E., Sarnow, P., Schneider, D., 2009. The Imd pathway is involved in antiviral immune responses in Drosophila. PLoS One 4 (10), e7436.
Doshch, S.F., Mahajan, S.D., Collins, A.R., 2009. SARS coronavirus spike protein-induced innate immune response occurs via activation of the NF-kappaB pathway in human monocyte macrophages in vitro. Virus Res. 142 (1–2), 19–27.
Du, L., Qin, L., Wang, X., Zhang, A., Wei, H., Zhou, H., 2014. Characterization of grass carp (Ctenopharyngodon idella) IL-17D: molecular cloning, functional implication and signal transduction. Dev. Comp. Immunol. 42 (2), 220–228.
Du, L., Feng, S., Yin, L., Wang, X., Zhang, A., Yang, K., Zhou, H., 2015. Identification and functional characterization of grass carp IL-17A/F1: an evaluation of the immunoregulatory role of teleost IL-17A/F. Dev. Comp. Immunol. 51 (1), 202–211.
Goubau, D., Deddouche, S., Reis e Sousa, C., 2013. Cytosolic sensing of viruses. Immunity 38 (5), 855–869.
Gunimaladevi, I., Savan, R., Sakai, M., 2006. Identification, cloning and characterization of interleukin-17 and its family from zebrafish. Fish Shellfish Immunol. 21 (4), 393–403.
Hansen, J.D., Zapata, A.G., 1998. Lympohcyte development in fish and amphibians. Immunol. Rev. 166, 199–220.
Hayden, M.S., Ghosh, S., 2008. Shared principles in NF-kappaB signaling. Cell 132 (3), 344–362.
He, L.B., Zhang, A.D., Pei, Y.Y., Chu, P.F., Li, Y.M., Huang, R., Liao, L.J., Zhu, Z.Y., Wang, Y.P., 2017. Differences in responses of grass carp to different types of grass carp reovirus (GCRV) and the mechanism of hemorrhage revealed by transcriptome sequencing. BMC Genomics 18 (1), 452.
Iwakura, Y., Ishigame, H., Saijo, S., Nakae, S., 2011. Functional specialization of interleukin-17 family members. Immunity 34 (2), 149–162.
Ji, J., Rao, Y., Wan, Q., Liao, Z., Su, J., 2018. Telost-specific TLR19 localizes to endosome, recognizes dsRNA, recruits TRIF, triggers both IFN and NF-kappaB pathways, and protects cells from grass carp Reovirus infection. J. Immunol. 200 (2), 573–585.
Jin, H.J., Shao, J.Z., Xiang, L.X., Wang, H., Sun, L.L., 2008. Global identification and comparative analysis of SOCS genes in fish: insights into the molecular evolution of SOCS family. Mol. Immunol. 45 (5), 1258–1268.
Kinger, L.M., Paul, S., Maynard, S.K., Cartwright, N.G., Schaefer, B.C., 2010. Cutting
