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Generation and functional evaluation of a DNA vaccine co-expressing Cyprinid herpesvirus-3 envelope protein and carp interleukin-1 beta

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ABSTRACT

Cyprinid herpesvirus 3 (CyHV-3) infection in carp causes a fatal and highly contagious disease that results in huge economic losses in common and koi carp aquaculture worldwide. Thus the development of an effective vaccine to protect carp stocks against the CyHV3 virus is imperative. In this study, we immunized common carps with a DNA vaccine consisting of a plasmid that co-expresses the CyHV-3 envelope protein ORF25 and the carp IL-1β gene in order to evaluate the adjuvant potential of IL-1β. Our result shows that antibodies specific to ORF25 can be detected as early as one week after intramuscular injection of the DNA vaccine at low dosage. Moreover, the co-expression of IL-1β can enhance the potency of the vaccine, as demonstrated by a higher antibody level after the third immunizations. Importantly, the DNA vaccine reduced mortality in carps when they were immunized prior to a CyHV-3 challenge, as compared to negative control groups. However, despite being able to induce higher neutralizing antibody titres, the co-expression of IL-1β in the DNA vaccine did not significantly improve the overall survival of immunized fish following virus challenge. Furthermore, the DNA vaccine can protect carps from tissue damage and histopathological alteration caused by viral infection. These strongly suggests that the vaccine can efficiently elicit protective immunity against CyHV-3 infection. In conclusion, the DNA vaccine formulated with the pIRES-ORF25-IL-1β DNA construct can protect carp against CyHV-3 infection and has potential applicability in the aquaculture industry.

1. Introduction

Cyprinid herpesvirus-3 (CyHV-3) is the causative agent of Koi herpervirus disease (KHVD). The manifestations of KHVD include skin graze, excessive secretion of mucus, apoptotic degeneration of multiple organs and sleepiness [1]. In 1996, CyHV-3 had been identified as the cause of a lethal infection of common carps and ornamental Koi, which has since then spread globally through international fish trade, Koi exhibitions as well as the practice of high density fish farming. Since common carp (*Cyprinus carpio carpio*) is one of the most cultivated food fish in Eurasia and ornamental koi (*Cyprinus carpio koi*) is a highly regarded pet fish, the global spread of CyHV-3 is incurring huge financial losses on the fish farming industry. The realization of this threat has finally prompted the World Animal Health Service or OIE to list KHVD as a reportable disease. The current epidemiological distribution of KHVD includes Israel, Indonesia, Japan, Germany, China, Canada and the United States [2–5].

CyHV-3 has other names, including Koi herpesvirus (KHV) and Carp interstitial nephritis and gill necrosis virus (CNGV). This gives, at times, confusing nomenclature. However, recent whole sequence analysis of the virus suggests close genetic relatedness to Cyprinivirus CyHV-1 and CyHV-2, therefore, CyHV-3 will be used in this report. CyHV-3 can infect various cell types and is most biologically active between 15 and 28 °C. At present, the genomes of four CyHV-3 strains isolated from Japan (KHV-1), China (KHV-GZ11), The USA (KHVU) and Israel (KHV-1) have been sequenced. The 295 kb dsDNA genome of CyHV-3 is predicted to encode 156 open reading frames (ORF).

Due to the economic impact of CyHV-3, several strategies have been employed to control the spread of the virus. One strategy involves exposing carps to CyHV-3 temporarily at 23 °C, an optimal temperature for viral replication, and subsequently transferring the infected carps to higher water temperature at 30 °C, in which the virus becomes inactive. In this way, carps that carry the inactive virus can generate adaptive immunity against the virus [6]. However, this intervention is costly and can routinely deplete 30–40% of the fish stock during operation. Furthermore, the practice can occasionally cause outbreak due to the release of the virus into the environment.

Alternatively, vaccination serves as an effective control strategy.

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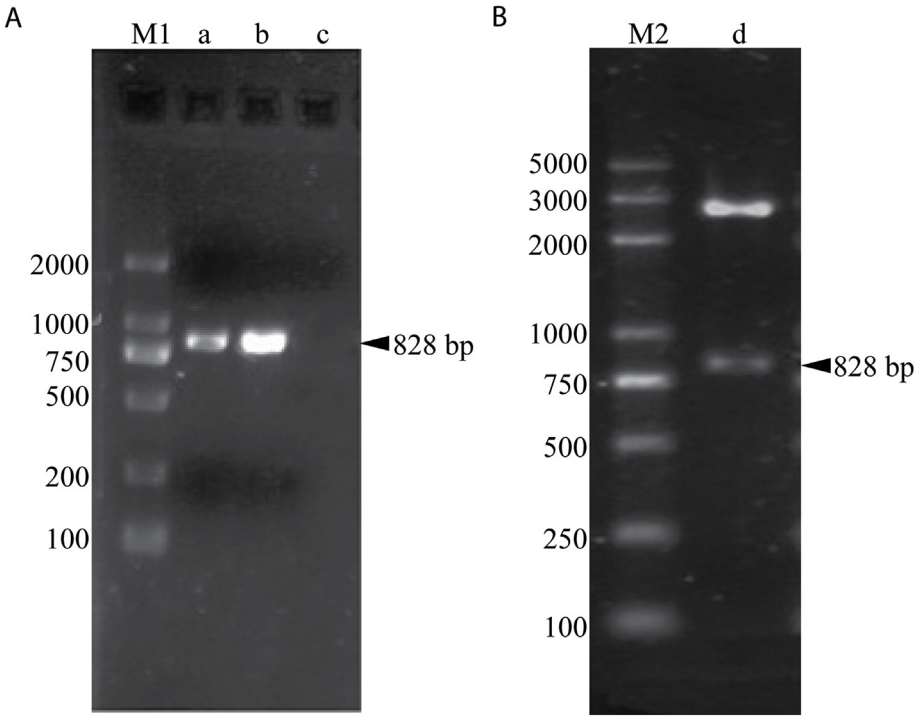

Fig. 1. Amplification of carp IL-1β.
Agarose gel electrophoresis of (A) Lane a–b: PCR amplification of IL-1β and lane c: negative control. (B) Lane d: restriction digestion of PMD18-T-IL-1β using Sma I and Xba I. M1: DL2000 DNA Marker, M2: DL5000 DNA Marker.

Existing mainstream vaccine commonly utilize weakened or inactivated virus as immunogens. Protection against the virus had been shown when fishes were immunized with ultra-violet irradiated CyHV-3 virus or when given an oral vaccine with liposomes encapsulated formalin-inactivated virus [7–9]. Furthermore, by manipulating well-defined genomic attenuations, several studies had managed to obtain non-virulent virus with the capability to still replicate in the host. These mutant virus are usually deleted with genes involved in the nucleotide metabolic pathways, such as ribonucleotide reductase, thymidine kinase, dUTPase and ORF12 [10,11]. Despite the well documented protection offered by weakened or inactivated virus, these methods are predisposed to a potential virulence reversion, especially when the virus are often persisting in the host population. Also, it is not easy to completely eliminate all residual virulence in these virus [10].

On the other hand, DNA-based subunit vaccines can circumvent the shortcomings of whole-agent vaccines. Li-Chun et al. demonstrated the applicability of using a DNA plasmid that allows the coexpression of a glycoprotein of spring viraemia of carp virus and the ORF81 protein of KHV in Lactobacillus plantarum as an oral vaccine formula. The vaccine was shown to protect 71% vaccinated carps and 53% vaccinated koi until 65 days post challenge [12].

The design of an effective DNA vaccine depends on the choice of the expressed antigens, which has to be immunogenic and exposed to the adaptive immunity. The availability of the CyHV-3 genome sequence has facilitated the screening of viral antigens as potential vaccine candidates. In our previous study, we investigated the potential of ORF25 as a vaccine candidate, which is a viral envelope protein and is predicted to be highly immunogenic. We have constructed a recombinant plasmid, pIRES-ORF25, in which the CyHV-3 ORF25 gene is placed under the control of a eukaryotic expression vector [13]. In this study, we aim to further enhance the efficacy of the ORF25-based DNA vaccine by co-expressing IL-1β as an adjuvant.

IL-1 was the first interleukin to have been characterized in bony and cartilaginous fish [14–16]. Subsequently, IL-1β becomes the first cytokine to be cloned from the Rainbow trout [17]. In fish, mounting data suggest that IL-1 serve an important role in the skin immune system, as well as regulating the expression of immune-related genes during infection [18,19]. IL-1 is also the first secreted pro-inflammatory cytokines upon infection in fish [20]. The gene structure of IL-1β in fish and in mammalian organisms are relatively conserved, both containing 12 anti-parallel β-strands forming a β barrel structure [21]. The recombinant IL-1β proteins are biologically active *in vitro* [22,23], with A number of studies clearly indicated the immune-stimulating effect of either injecting IL-1β DNA or the recombinant proteins into fishes [24,25]. However, while the potential benefits of using IL-1β as a vaccine adjuvant have been emphasized, there is no report of using IL-1β as an adjuvant in DNA vaccine developed for aquaculture use.

In the present study, we aim to improve and modify our ORF25-based DNA vaccine. We generated a DNA plasmid that co-expresses CyHV-3 ORF25 and ectopic IL-1β. Using the common carp as a model, we evaluated the efficacy of the vaccine after intramuscular injection to carps and assessed the potential of IL-1β as vaccine adjuvant. Here, we show that the modified DNA vaccine can rapidly increase the production of antigen-specific antibody in carps after immunization, but not in the enhancement of *in vivo* protection against virus challenge. Our study provides important reference on the potential of using IL-1β as an adjuvant for fish DNA vaccine.

2. Result

2.1. Amplification of IL-1β gene and generation of pMD18-T-IL-1β

In order to design a DNA vaccine that co-expresses IL-1β as an adjuvant, we sought to clone the native IL-1β coding sequence from carp. We obtained total RNA from carp tissue and reverse transcribed the RNA to obtain total cDNA. The total cDNA was then used as the template for amplifying the full-length IL-1β coding sequence using a specific pair of primers. SmaI and XbaI sites were added to the primers. A DNA fragment with size of around 830bp could be observed in the agarose gel following gel electrophoresis (Fig. 1A), the size is expected