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Comparative study of the immunoprotective effect of two DNA vaccines against grass carp reovirus

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ABSTRACT

Grass carp reovirus II (GCRV II) causes severe hemorrhagic disease with high mortality in grass carp, *Cyenopharyngodon idellus*. DNA vaccination has been proven to be a very effective method in conferring protection against fish viruses. However, DNA vaccines for GCRV II have not yet been conducted on grass carp. In the current work, we vaccinated grass carp with a DNA vaccine consisting of the segment 6 (pC-S6; encoding VP4) or 10 (pC-S10; encoding NS38) of GCRV II and comparatively analyzed the immune responses induced by these two vaccines. The protective efficacy of pC-S6 and pC-S10, in terms of relative percentage survival (RPS), was 59.9% and 23.1% respectively. This suggests that pC-S6 and pC-S10 DNA vaccines could increase the survival rate of grass carp against GCRV, albeit with variations in immunoprotective effect. Immunological analyses indicated the following. First, post-vaccination (pv), both pC-S6 and pC-S10 up-regulated the expression of interferon (IFN-1), Mx1, IL-1β, and TNF-α. However, CD4 and CD8α were up-regulated in the case of pC-S6 but not pC-S10. Second, comparing non-vaccinated and pC-S10-vaccinated fish, the T cell response related genes, such as CD4, CD8α, and GATA3, were elevated in pC-S6-vaccinated fish at 48 h post-challenge (pc). Third, pC-S6 and pC-S10 induced similar patterns of specific antibody response pv. However, only anti-VP4 IgM in the sera of surviving fish infected with GCRV was significantly increased pc compared with that pre-challenge. Taken together, these results indicate that pC-S6 promotes both innate (IFN-1 and Mx1 induction) and adaptive (T cell and specific antibody response) immunity pv and that the induction of a memory state promptly primes the immune response upon later encounters with the virus, whereas pC-S10 only induces the type I IFN-related response pv and a lower inflammatory response pc.

1. Introduction

Grass carp reovirus (GCRV) is a double-stranded RNA virus belonging to the genus *Aquareovirus* (AQRV), family *Reoviridae* [1], which causes severe hemorrhagic disease with approximately 85% mortality in fingerling and yearling grass carp, *Ctenopharyngodon idellus*, in China [2]. It can be divided into three genotypes (GCRV I, II, and III) based on genomic and biological characteristics, among which the gene sequence identity is less than 20% [3]. Epidemiology analyses have shown that the three genotypes exist simultaneously in China and that the most common isolates belong to GCRV II [3]. Several GCRV II isolates from different areas of China have been completely sequenced, and their protein sequences share 95.3–99.4% identity [4]. The genomic RNA of GCRV II, which contains 11 segments (S1−S11), is predicted to encode 11 proteins, including three inner core proteins (encoded by S1, S2, and S3), two nonstructural proteins (encoded by S4 and S10), four capsid proteins (encoded by S5, S6, S9, and S11), one fiber protein (encoded by S7), and one unknown protein (encoded by S8) [4].

DNA vaccination has proven to be a very effective method in conferring protection against fish viruses, such as rhabdoviruses [5–10] and GCRV I [11,12]. The immune response following IHNV DNA vaccination has been separated into three distinct and inter-related phases: the early, specific, and long-term antiviral responses [7,13]. It has also been temporally and spatially segregated: the injection site (local),

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Table 1
Primers used in this study.

Gene	Primer Sequence[a]	Accession No.
GCRV-S6	F: 5′-CTAGGATCCATGGGAAACGTCCAGACGAAC-3′ R: 5′-CCGCTCGAGCTAAGACGGAGGAGGCCAGTATC-3′	GQ896337.1
GCRV-S10	F: 5′-CGCGGATCCATGGCGGGTGTGTCTCTCAAC-3′ R: 5′-CGCTCGAGCTACAGCATCTGCGCGAATATCCGTCT-3′	GU350747.1
S6	F: 5′-GCTGATGCTGCAGACGGCTAAAC-3′ R: 5′-TAATTGCCTGCTGCGCTGACT-3′	GQ896337.1
S10	F: 5′-TACTGTGCAACCCTTATTGGTGGC-3′ R: 5′-CATCTGCGCGAATATCCGTCTTACC-3′	GU350747.1
β-actin	F: 5′-CCCAAAGCCAACAGGGAAAAGA-3′ R: 5′-GGCAGGGCATAACCCTCGTA-3′	M25013
IFN-1	F: 5′-AAGCAACGAGTCTTTGAGCCT-3′ R: 5′-GCGTCCTGGAAATGACACCT-3′	DQ357216
Mx1	F: 5′-CTGGGGAGGAAGTAAAGTGTTCT-3′ R: 5′-CAGCATGGATTCTGCCTGG-3′	HQ245104
IgM	F: 5′-GAGGCATCGGAGGCACATTTC-3′ R: 5′-TTGGGTCTCGCACCATTTTCTC-3′	DQ417927
CD4	F: 5′-CAAATCAAGCATTATGGAAGTGC-3′ R: 5′- ATAGGATGAGGAGAGAGAGGTA-3′	GQ355588
CD8α	F: 5′- AAGGAGAACCAGATCCAACAAC-3′ R: 5′- AGAATGAGGAGAAGAGCACAGC-3′	GQ355586
T-bet	F: 5′-CTCTAACAATGTAGGACAGATGATA-3′ R: 5′-GGTAGGCAGTGACGGCAATGA-3′	JX021296
IFN-γ2	F: 5′-GATGACTTTGGGATGGATGACA-3′ R: 5′-CTGTTCACTTTCCTCAAGATTCA-3′	JX196701
GATA3	F: 5′-TCCAGCCCACACCTCTTCAC-3′ R: 5′-GATAGAAGCCATGCTCCGACTA-3′	JX021295
IL-4/13B	F: 5′-CAAGCAGCAAAGGTCCTGAATG-3′ R: 5′-TCACTGGATGTTCCTCTGAAGC-3′	KP896505
IL-1β	F: 5′-TGTGACGCTGAGAGACGGAAA-3′ R: 5′-GAGTTTCAGTGACCTCCTTCAA-3′	JX014320
TNF-α	F: 5′-CCATCCATTTAACAGGTGCATAC-3′ R: 5′-CAGCAGATGTGGAAAGAGACC-3′	HQ696609

[a] Restriction sites for plasmid construction are underlined.

kidney and spleen (systemic), and possibly gills (mucous) [7]. The early response is non-specific and transient and will cross-protect against infection from other viral species, although not against bacterial pathogens [10,14,15], suggesting that type I interferon (IFN)-like activity is responsible [16]. After that, in the later stage, both humoral (antibodies) and cellular ($CD4^+$ and $CD8^+$ T cells) immune responses are induced, at which point fish are specifically protected only against the virus for which they are DNA vaccinated [17–19]. Finally, the long-term antiviral response has also been described, suggesting that IgM^+ memory B cells and/or additional factors such as cellular immunity are likely to be involved, but this requires further study [13,20]. In addition, the immune response varies depending on the antigen, the dosage of the vaccine, and the temperature [21,22]. However, the immune mechanisms of DNA vaccines for non-rhabdoviruses are still not completely understood.

Apart from the immune response induced by the DNA vaccine, the immune response to virus infection in fish vaccinated previously is also of great relevance to the mechanism responsible for protection, since neither the humoral nor cellular immune responses induced following DNA vaccines always correlate with *in vivo* protection levels [16]. Regarding the immune response to the virus in vaccinated fish or non-vaccinated fish, previous reports have suggested that DNA vaccination induces a memory state in fish that primes non-specific immune responses upon later encounters with the virus [23]. On the contrary, different types of adaptive responses are prominent, affected by the site and stage of infection [24]. Nevertheless, the results suggest that different responses to infection are clearly observed when comparing vaccinated and non-vaccinated fish. Therefore, focusing on the secondary immunity response of DNA-vaccinated or non-vaccinated fish elicited by boost or virus in relation to the primary immunity response may elucidate the regularity and mechanism of immunity and offer guidance on a “prime-boost” strategy.

So far, only one recombinant protein subunit vaccine with protection against GCRV II has been described [25]. As such, in this study, we generated two DNA vaccines consisting of S6 and S10 of GCRV II, respectively. Through *in vivo* studies, grass carp were intramuscularly injected with the plasmids, and the immunoprotective effects and immune responses were undertaken. Results were analyzed to elucidate the protective mechanisms conferred by these vaccines from three aspects: the response induced by the DNA vaccine, the association between the vaccination and the infection, and the relationship between the primary and the secondary immune response. Comparisons of the mechanisms of these two vaccines were also performed.

2. Materials and methods

2.1. Fish

Grass carp (15–20 g) obtained from Wulonggang Aquatic Product Development Company (Guangdong Province, China) were maintained at Xiantao Fishery (Hubei Province, China) at about 28 °C and fed daily with a commercial diet. Prior to the vaccination experiments, fish were acclimatized for 2 weeks and then randomly sampled from the liver, kidney, and spleen for the examination of the virus by RT-PCR.

2.2. Virus and in vivo viral titration

GCRV-HF was isolated from diseased grass carp (Hefei, Anhui Province, China) and purified according to the previous report [4]. The virus was confirmed to belong to GCRV II by a duplex PCR [26]. Virus stock was titrated by *in vivo* infection experiments and the amount used for challenge experiments was that required to achieve mortality above 90% in 14 days.