Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage:www.elsevier.com/locate/apjtd

Document heading doi:10.1016/S2222-1808(12)60018-8

Deep insight into white spot syndrome virus vaccines: A review

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ARTICLE INFO

Article history:
Received 8 July 2011
Received in revised form 23 August 2011
Accepted 14 November 2011
Available online 28 February 2012

Keywords:
Virus
Vaccines
Diseases
Shrimps
Infections
WSSV

ABSTRACT

White spot syndrome virus (WSSV), the causative virus of the disease, is found in most shrimp farming areas of the world, where it causes large economic losses to the shrimp farming industry. The potentially fatal virus has been found to be a threat not only to all shrimp species, but also to other marine and freshwater crustaceans, such as crab and crayfish. To date, no effective prophylactic treatment measures are available for viral infections in shrimp and other crustaceans. Due to current aquaculture practices and the broad host range of WSSV, intervention strategies including vaccination against this virus would be pivotal to save and protect shrimp farming. Several achievements have been attained in the search of novel vaccines for WSSV. DNA vaccination, recombinant vaccines, oral vaccination techniques and gene therapy are some of the thrust areas of focus for scientists and researchers. This review article highlights the recent trends in the development of WSSV vaccines either as DNA vaccines or recombinant vaccines and their functioning strategies as suggested by the researchers worldwide.

1. Introduction

In many countries aquaculture is a major thrust area which plays vital role in improving community progress, food security, poverty mitigation, employment and other economic activities[1]. Sustainable improvement of aquaculture relies on disease avoidance. With a strengthening of operations, the risk of disease occurrence and stretch of infectious diseases increases. There is an insightful and reliable general positive outlook towards vaccines. Vaccines stimulate the immune system to help fight off diseases and the application of these methods to control infectious diseases is growing in importance. Vaccines kindle the immune system to help fight off diseases and the application of these methods to control infectious diseases is growing in significance[2]. Perfecting the use of adjuvant and delivery systems is needed to meet the demand for vaccines in order to ensure the safe supply of healthy fish products. The production of vital species of cultivated penaeid shrimp has increased exponentially since the early 1970s[3]. Viruses are the simplest forms of life yet they play a crucial role in regulating planetary processes. They are major components of the marine food web affecting bacteria, archaea and eukaryotic organisms, with consequences for nutrient and energy cycling, control of species diversity and exchange of genetic material among organisms in marine environments. It is estimated that as much as 10-20% of marine bacteria is lysed daily with 2-3% of primary production lost through viral activity. Thus the viruses play a key role in energetic and as the largest reservoir of genetic diversity in the marine environment. A serious of recent studies has shown that viruses help in manipulating the life histories and understanding the hosts in remarkable ways, challenging our understanding of the almost invisible world of viruses[4,5]. Viruses are among the most critical pathogens affecting crustaceans, especially shrimp. Viral diseases were found most demoralizing in a global point of view. So far 20 shrimp viruses have been reported[6,7]. Major viral pathogens of shrimps include white spot syndrome virus (WSSV), monodon baculo virus (MBV), yellow head virus (YHV), Infectious hypodermal and hematopoietic necrosis virus (IHHNV), hepatopancreatic parvo virus (HPV), taura syndrome virus (TSV), baculovirus penaei (BP) and baculo virus midgut gland necrosis virus (BMNV)[8].

WSSV, the pathogen of shrimp white spot disease, is a rod-shaped enveloped double-stranded DNA virus belonging to Nimaviridae family[9]. WSSV is type species of the genus *Whispovirus* of the new family Nimaviridae. WSSV is a large double-stranded DNA containing virus with an ovoid to bacilliform shaped virion containing one rod-shaped nucleocapsid and replicates in the nucleus.

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In addition to the use of *in situ* hybridization techniques, polymerase chain reactions (PCR) and immunological detection methods have been developed for the detection of WSSV. It causes up to 100% mortality within 3 to 10 days of infection, resulting in major economic losses to the shrimp farming industry[10-14]. WSSV infects a wide range of aquatic crustaceans, including crabs, lobsters, and freshwater crayfish[15]. WSSV is extremely virulent, possesses a wide range of host specificity and targets various tissues. The virus is pathogenic to several species of shrimp, such as black tiger shrimp, *Penaeus monodon* (*P. monodon*), kuruma shrimp and *Metanephrops japonicus*. The portals of entry of virus have not yet been clearly defined[16]. Li *et al*[17] described the cell surface molecule integrin as a cellular receptor for WSSV entry.

The first major WSSV outbreak, reported in 1993, resulted in a 70% reduction in shrimp production in China[18,19] and this virus has remained a major concern for shrimp aquaculture throughout the world since. The presence of WSSV has been reported in both wild and hatchery reared post larvae[15,20]. WSSV has become an epizootic disease and is not only a major threat to shrimp aquaculture, but also to marine ecology[21]. Epidemiological data has been reported for WSSV indicating that the presence of the virus does not necessarily result in white spot disease (WSD). It has been shown that if the risk is minimized then the disease can be avoided or reduced. Thus, successful shrimp crops can be harvested when WSSV and other shrimp viruses are present at low viral prevalence[22], when stress is reduced or when the virus is detected early enough, outbreaks can be prevented despite the presence of WSSV. The characteristics of common viruses affecting shrimps are listed in Table 1. WSSV-infected shrimp may rapidly develop white spots (ranging from 0.5-3.0 mm in diameter) on the exoskeleton, appendages and inside the epidermis. Since these spots are not always present, and since similar spots can be produced by some bacteria, high alkalinity and stress, they are not considered a reliable sign for preliminary diagnosis of this disease. Other signs of WSSV include lethargy, sudden reduction in food consumption, red discoloration of body and appendages and a loose cuticle[23].

It has been reported that sick and dying shrimp lead to rapid progression of WSD and there is increasing evidence that the ingestion of sick or dying shrimp is the major mode of transmission[24]. Organs of ectodermal and mesodermal origin are the main target of WSSV. This includes epidermis, gills, foregut, hindgut, antennal gland, lymphoid organ, muscle, eye-stalk, compound eye, heart, gonads, body cuticular epithelium, hematopoietic cells, and cells associated with nervous system, pleopods, pereiopods, testes and ovaries[25,26]. Quantitative pathogenic analyses suggest that the major target tissues for replication are gills, stomach and body cuticular epithelium, hematopoietic tissues, lymphoid organ and antennal glands[26]. Attenuated virus contains bacteria or viruses that have been altered so they can not cause disease. Killed Vaccines contain killed bacteria or inactivated viruses. Toxoid vaccines contain inactive toxin bacterial (only applied in vertebrates).

Table 1

Differential diagnosis of virus-induced mortalities that may occur in 4 major viral diseases in shrimp farms.

Activity	WSSV	IHHNV	TSV	YHV
Stage of grow out	All	All	2-6 weeks of post stocking	7-10 weeks post stocking
Mortality	High rapidly increasing	High rapidly increasing	moderate	High rapidly increasing
External appearance	White spots in the body and red discoloration	White spots in two three segments	Red discoloration	Yellow and pale discoloration
Organs affected	Subcuticular epithelium, connectiove tissue,gills, lymphoid organ.	Gills, lymphoid organs, epidermis and hypodermis, connective tissue, intestines. nerves	Subcuticular epithelium, connective tissue, gills	Subcuticular epithelium,gills, lymphoid organ
Inclusion bodies	Intranuclear	Intranuclear	Intracytoplasmic	Intracytoplasmic basophilic

Recombinant vector vaccines are experimental vaccines similar to DNA vaccines, but they use an attenuated virus or bacterium [27]. DNA Vaccines contain genes into a vector to produce a microbe's antigens and introduced into the body. The body's own cells become vaccine-making factories, creating the antigens necessary to stimulate immune system[28,29]. RNAi Vaccines contain dsRNA from viral specific gene (sometimes endogen gene) and applied for intramuscular injection induces an antiviral activity response for blocking of respective gene[30,31]. There are a lot of studies for preventing and controlling shrimp WSSV infection, such as improvement of environmental conditions, induction of non-specific antiviral response with antivirus drugs or immunostimulants[32-35], neutralization antibodies[34,36,37] and suppression of virus by RNAi technology[38,39]. To date, two kinds of "vaccines", protein and DNA, have been used to protect against crustacean WSSV. VP19, VP26, VP28, and VP292 of WSSV were used as protein vaccines, and VP28, VP281, VP15, and VP35 as DNA vaccines. Some of these subunit vaccines, such as VP28, have been shown to efficiently protect shrimp or crayfish against WSSV. However, VP19 showed different functions against WSSV in different studies [40].

2. DNA vaccines

Outbreak of disease is one of the major stumbling blocks in the development and sustainability of aquaculture. A number of approaches have been made in attempts to solve disease problem in aquaculture, one among these is vaccination[41]. WSSV is a large DNA virus with five major proteins with expected sizes of 28 kDa (VP28), 26 kDa (VP26), 24 kDa (VP24), 19 kDa (VP19) and 15 kDa (VP15). VP28 and VP19 are associated with the virion envelope and the others are associated with the nucleocapsid [42,43].Subunit vaccines targeting envelope proteins VP28 and/or VP19 expressed in *E. coli* have been studied and their protective ability against WSSV infection by oral administration[44] or intramuscular injection[45]. DNA vaccination is the process in which naked-DNA coding for a required antigen, and not the final

antigen itself, is presented to the animal to be protected. This naked DNA, usually presented as a plasmid, is translated by the host cell into the immunogenic protein and expressed on the cell surface. The presence of a pathogen antigen in conjunction with host cell surface molecules will potentially trigger an effective immune response against the antigen[46,47]. Yu Mi Ha *et al* concluded that the envelope protein, VP19 of WSSV plays a critical role in WSSV infection to shrimp[48].

Gene therapy can be defined as the delivery of a therapeutic gene for expression in somatic tissue. There has been a rapid development in the field of gene therapy and DNA vaccination since expression of foreign genes *in vivo* was demonstrated. Subsequently, it was known that injection of naked plasmids into the muscle could also elicit an immune response. DNA vaccines do not need the gene to be permanently expressed as a transient expression of the gene is sufficient for evoking the immune response. More recently, DNA vaccines encoding envelope proteins VP15, VP28, VP35 and VP281 of WSSV were developed and tested in black tiger shrimp[28]. According to recent studies it is estimated that WSSV is assembled by at least 59 structural proteins, among them 35 are envelop protein and 9 are nucleocapsid [6]. The potential of DNA based vaccines against viral diseases in aquaculture has proven to be effective to reach a sustainable production.

Recent studies have reported the protection conferred (for up to 50 days) to experimentally WSSV-infected shrimp *P. monodon* injected with recombinant DNA plasmids encoding the envelope proteins VP28 or VP281, while the injection of DNA expressing the WSSV nucleocapsid proteins VP15 and VP35 did not elicit a protective response. Besides, it was found that shrimp vaccinated with recombinant DNA showed a longer protection effect (up to 50 days), whereas organisms vaccinated with the envelope protein VP28 were protected for a shorter period(upto 14 days)[28]. Furthermore, those organisms vaccinated with recombinant DNA encoding VP28 showed enhanced levels of prophenoloxidase and superoxide dismutase, suggesting that these immunological parameters may contribute to confer resistance to shrimp against WSSV[49].

3. Oral and recombinant vaccination

Choi *et al*[33] carried out a study for the molecular level identification of recombinant protein vaccine efficacy, by oral feeding against WSSV infection, with the comparison of viral mRNA transcriptional levels in shrimp cells. Viral structural proteins, especially the envelope proteins, are important because of not only virion morphogenesis but also that they are the first molecules to interact with the host cell[32]. The structural proteins often play vital roles in cell targeting, and virus entry, assembly, and budding[50]. Among the structural proteins, viral protein 28 (VP28) expressed in the outer surface of WSSV facilitates the entry of the virion into the cell at the early WSSV infection stage[51]. VP28 is on envelope protein on the surface of the WSSV and reacts with anti-WSSV polyclonal antibodies. This suggests that recombinant VP28 (rVP28) could be a common antigen to control white spot diseases of shrimp[42,43,52]. Choi *et al* evaluated the vaccination effects on mRNA transcription of WSSV genes by the administration of the recombinant viral proteins, rVP19 and rVP28, as oral protein vaccines. VP19 and VP28 of WSSV are major structural proteins that play key roles for virus attachment and penetration into host cell in the life cycle of the virus[53]. These structural proteins form a multiprotein complex with other viral proteins such as tegument protein VP26, nucleocapsid protein VP24, and envelope protein VP51A for virus assembly and penetration into host cell[53,54]. The shrimp immune system was able to recognize WSSV structural proteins, and studies showed that the vaccination of shrimp against WSSV can be made possible by the oral feeding of the protein vaccines rVP19 and rVP28.

But recently, the DNA vaccines encoding envelope proteins like VP15, VP28, VP35 and VP281 of WSSV were developed and tested in black tiger shrimp[28]. The report suggested that DNA vaccination using expression vectors encoding VP28 and VP281 has potential to increase protection against WSSV infection. However, the immune responses stimulated in shrimp by DNA vaccination have not been studied thoroughly to date. Recently, it was reported that primary vaccination with WSSV recombinant VP26 and VP28 showed recovery of the resistance against WSSV infection[55]. This result suggests that the prime boost immunization with DNA vaccine will extend the duration period of DNA vaccine. The use of oil emulsion as adjuvant in this effort may cause major drawbacks as some fishes and shellfishes show unacceptable levels of side effects. In this context, use of nanoparticle carriers like chitosan and poly-lactide-co-glycolide acid (PLGA)[56] of vaccine antigens together with mild inflammatory inducers may give a high level of protection to fishes and shellfishes not only against bacterial diseases, but also from certain viral diseases with vaccine-induced side effect. At the same time, these results may suggest that the immune responses of vaccinated shrimp against WSSV are weak and not lasting for long period because shrimp does not have memory cells like mammals. Treatment of WSV is not an option thus the detection is very vital. A quick response and damage control are required in order to prevent the spreading of disease. Thus, early detection of sick/dying shrimp (monitoring numbers), use of pond side diagnostics and safe disposal of dying shrimp reduce the impact of WSD[57].

4. Conclusion

The safety of DNA vaccines for use in shrimps is more of a concern than their efficacy. Safety issues are related to integration into chromosomal DNA, pathological processes at the site of injection distribution to internal organs and longevity of retention of foreign DNA in these organs. Issues related to tumourigenicity will probably raise public concern and potentially also with the regulatory bodies. The use of some innate immune-related genes as biomarkers may enable us to know whether vaccines will be an effective tool to combat against pathogens. Biosecurity can be defined as the concept of protecting cultured shrimps from contamination by diseases and of preventing the spread of diseases[58,59]. WSSV had spread to all shrimp growing nations and got recognized as the most serious pathogen of crustaceans[7,16,60-64]. Biological, scientific and technical restrictions presently still prevent the generation or commercialization of vaccines for all economically significant fish diseases. A better understanding of WSSV structural proteins and the localization in the virion will

shed more light on virus assembly, its infection pathway and the discovery of antiviral drugs. In the future the world shrimp industry will be overwhelmingly dominated by cultivation of domesticated lines of shrimps. Most of the stocks used will also be improved by genetic selection for growth rate and other desirable traits like disease tolerance. *P. vannamei* stocks are highly successful when reared with good biosecurity and good management of feed and the pond environment. The latter can be achieved by following good aquaculture practices recommended by the Global Aquaculture Alliance. Nucleic acid-based vaccines seem to be a very promising and valuable approach against WSSV in the short-term. However, an important step in the rational design of a vaccine is to understand the immune correlates of protection. Unfortunately, the field of invertebrate immunology is severely delayed when compared to that of vertebrates, although the mechanistic understanding of the invertebrate immune system is rapidly evolving. Also the field of marine viruses and the interactions with their respective hosts is still at a relatively early stage, which gives valuable and exciting opportunities for research.

Conflict of interest statement

We declare that we have no conflict of interest.

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