Development of a gene-fusion subunit vaccine against porcine reproductive and respiratory syndrome virus

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1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is the causative pathogen of porcine reproductive and respiratory syndrome (PRRS), one of the most important diseases in the swine industry (Beyer et al., 2000). PRRS was first described in the late 1980s in North America and then has spread globally with an estimated loss of USD 664 million annually in the swine industry in the USA (Holtkamp et al., 2012). Approximately two million pigs were infected with PRRSV and highly pathogenic PRRSV (HP-PRRSV) per year (Tian et al., 2007). The development of safe and effective vaccines against PRRS infection has been considered as an important approach in the prevention and control of this disease.

2. Design Concept

In this technical platform, the baculovirus-insect cell expression system was used for the production of a novel subunit vaccine against porcine reproductive and respiratory syndrome virus (PRRSV). Baculovirus expression systems produce high levels of recombinant proteins and carry out complex posttranslational modifications. PRRSV is an enveloped virus which belongs to the *Arteviridae* family (Snijder et al., 1998). The genome of PRRSV consists of a single-stranded, 5′capped and 3′-polyadenylated mRNA molecule that is 15 kb in size. The viral genome contains two large open reading frames and seven smaller open reading frames (ORFs): ORF 1a and 1b code for the viral replicase, and ORF 2 to 7 for structural proteins (Meulenberg et al., 2000). In this technical platform, a nucleotide sequence of a mutated gene fusion containing two ORFs of PRRSV was designed to increase the antigen protein yield and enhance the immunogenic activity against PRRSV. The mutated gene fusion was constructed and cloned into a transfer plasmid vector. The recombinant plasmids were co-transfected into insect cells with a baculovirus vector to produce recombinant baculovirus. Expressed fusion proteins were confirmed by using SDS-PAGE and western blotting. The antigenicity of the protein vaccine was analyzed using animal study and its potential as a vaccine candidate against PRRS was evaluated.

3. Technical Development

In this technical platform, a mutated gene fusion designed for subunit vaccine against PRRSV was synthesized and cloned into a transfer plasmid vector. The transfer plasmid vector harboring fusion gene construct was co-transfected into insect cells with linearized virus DNA and culture medium was harvested. The culture medium with recombinant baculovirus was used to amplify further stock of recombinant baculovirus. Insect cells were infected with the recombinant baculovirus at an appropriate multiplicity of infection and harvested the cells for analysis of the recombinant proteins by SDS-PAGE and Western blotting. Total protein level was determined by Bradford method. For preparation of subunit vaccine, recombinant protein antigens were mixed with adjuvant. The piglets were vaccinated with the subunit vaccine to evaluate the antigenicity. The results showed that the subunit vaccine could protect the piglets from PRRSV infection. Thus, the subunit vaccine has potential as a vaccine candidate against PRRS.

Fig. 1 Eletrophoresis analysis of the recombinant baculvirus DNA containing mutated fusion gene in 1% agarose gel.

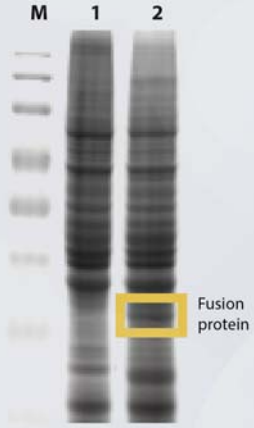

Fig 2 SDS-PAGE analysis of fusion antigen proteins.

4. Technological Competitiveness

The worldwide problem of PRRSV infection has necessitated the development of an effective vaccine. The commercial vaccine products available for the prevention of PRRS included attenuated live vaccines and inactivated vaccines. Attenuated live vaccines can potentially revert into a virulent and dangerous one whereas inactivated vaccines were safer but may not offer significant protection. To develop a safe and effective vaccine against PRRSV, a novel subunit vaccine has been designed and expressed using baculovirus-insect cell expression system. In this technical platform, a nucleotide sequence of a mutated gene fusion containing two ORFs of PRRSV was cloned and expressed successfully in insect cells. This system has achieved high levels of expression and can be easily scaled up to large volume insect-cell bioreactors. Otherwise, animal test in pigs has proven that this novel subunit vaccine is capable of protecting 100% of the vaccinated animals from PRRSV infection. In conclusion, an effective subunit vaccine has been successfully developed by the baculovirus-insect expression system. The high production yields and safety would make it possible for its use as a commercial vaccine.

5. R&D Result

In this technical platform, we describe the development of an inexpensive, effective, and safe subunit vaccine against PRRSV. To increase the antigen protein yield and enhance the immunogenic activity against PRRSV, a nucleotide sequence of a mutated gene fusion harboring two ORFs of PRRSV was synthesized and expressed in insect cells. SDS-PAGE and western blotting confirmed the expression of antigen proteins. The antigenicity of the subunit vaccine was verified using animal test in pigs. Otherwise, quantitative assay showed that high production yields of protein antigens were obtained using this technical platform. In conclusion, the technical platform offers a mean for high yield and economical production of an immunogenically active subunit vaccine against PRRSV infection.

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