

Vaccine Design and Therapeutic Efficacy of African Swine Fever

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Abstract: African swine fever (ASF) refers to an infectious disease caused by infection of ASF virus (ASFV) in pigs, which is characterized by high infectivity and high mortality rate. There is no complete cure for ASF, and relevant vaccines are still under development. This article will discuss the vaccine design and treatment efficacy of ASF. Subunit vaccines and inactivated vaccines are currently being improved and applied, which have better prospects for application. Subunit vaccines are relatively safer because they only contain a small part of the ASFV. Vaccines can be optimized by researching the protein composition of ASFV, where p72 protein, p54 protein, p22 protein and p49 protein are key proteins in ASFV replication. These proteins jointly regulate the replication of ASFV. Studying the antigenicity of monoclonal antibodies designed based on different proteins of ASFV can help optimize vaccines. The p49 protein has certain antigenicity. The p22 protein plays a role in the replication process of the virus. The p72 protein, as a capsid protein, plays a key role, so vaccines can be designed based on the p72 protein. The combined use of inactivated vaccines and subunit vaccines can help ASF vaccines take effect. Studying these key proteins can help improve the design of ASF vaccines, enhance antigenicity, and lay a foundation for solving this major problem.

Keywords: African swine fever, vaccine development, vaccine efficacy, therapeutic mechanism

1. Introduction

African swine fever (ASF) refers to a highly contagious infectious disease caused by infection of ASF virus (ASFV) in domestic pigs or wild boars. It has a very high infectivity and the clinical manifestations are characterized by high fever, lethargy, anorexia, hemorrhage in various organs. ASF mainly occurs in the regions south of the Sahara, Sardinia of Italy, the Caucasus region, as well as some countries in Russia and Eastern Europe. It has a significant impact on social economy and the environment, posing a serious threat to the global pig industry and many related stakeholders. This disease is classified as a legally reportable animal disease by the World Organization for Animal Health (OIE), and currently, no drug can completely cure ASF. The transmission mode is that soft ticks are infected by biting wart pigs carrying the virus, and then the virus is transmitted by biting susceptible pigs. A large number of studies have shown that antibodies produced by ASFV can play a certain role, but the cytotoxic T lymphocytes induced by it may be a crucial factor for the body to fully exert its protective effect.

The shape of ASFV is pentagonal or hexagonal, with a size of 175-215 nm. The viral nucleocapsid protein is 20-faced symmetrical, with a diameter of 180 nm, and the genome is composed of a single-stranded linear double-stranded DNA with a length of 170-190 kb. The DNA molecule has a covalently closed terminal, inverted repeat region and hairpin structure, and 100 or more viral proteins can be detected in infected cells, among which the main immunogenic and diagnostic proteins include p72, p54 and p30. The ASFV genome is frequently mutated and has genetic diversity [1]. The encoded enzymes and proteins in the genome participate in regulating the relationship between the virus and the host, and play an important role in virus replication, survival and transmission, and persistent infection. Only about 68 structural proteins have been identified, including pp220, pp62, p72, p54, p22, p11.5, p12, p17, p10, p14.5, p150, p37, p34, p14, and CD2v proteins. Due to the presence of various viral proteins in ASFV and its antigenicity, vaccines can be designed based on proteins. ASF vaccines include inactivated vaccines, nucleic acid vaccines, subunit vaccines, and live virus vector vaccines. Regarding the study of ASFV proteins, the first is p22 protein. The p22 protein is encoded by the KP177L gene and is the main structural protein of ASFV. The p22 protein is an early protein located on the external surface of the virus particle. Immunizing pigs with the p54 and p30 proteins of ASFV expressed by baculovirus can induce the production of neutralizing antibodies, and some pigs can resist the attack of strong virulence strains.

When pigs are immunized with p30, p54, p72, and p22 proteins expressed by baculovirus simultaneously, although high-titer neutralizing antibodies with neutralizing activity can be induced, they cannot resist the attack of strong virulence strains. The p22 protein is a viral transmembrane structural protein transcribed early after infection with ASFV and located on the inner membrane of the ASFV particle and the surface of the infected cell. Through research, it has been known that the p22 protein of ASFV can interact with several host proteins involved in different cellular pathways, which are helpful for the endocytosis process of the viral infection. The p22 protein plays a key role in the replication process of the virus. As the main structural protein of ASFV, the p22 protein has high research value and has laid the foundation for the subsequent design of subunit vaccines based on it. The p72 protein is also a major structural protein. P72 is produced in the late stage of viral infection and is the main capsid protein of ASFV, encoded by the B646L gene. The p49 protein is also crucial. The p49 protein is a capsid protein encoded by the B438L gene and is expressed in the late stage of infection. It is a structural protein necessary for the infection of virus particles. When p49 is lacking, although the virus particles formed by the virus have a tubular structure, the icosahedral symmetry is lost. P49 is located near the apex of the capsid, indicating that this protein plays an important role in constructing or stabilizing the icosahedral apex of the virus particles. Regarding subunit vaccines, it has been confirmed that multiple viral proteins can induce neutralizing antibodies. Compared with attenuated live vaccines, vaccines based on antigens and single or partial genes have higher safety. Some proteins related to the attachment and internalization of ASFV, such as p54, p30, p72, and CD2v, have been used in the development of subunit vaccines. This article aims to conceive feasible vaccines based on ASF proteins. It also covers the current research status and application of ASFV. Some of the main protein structures and functions of the ASFV will be introduced.

2. Structure and composition of ASFV

ASFV is the only known double-stranded DNA large enveloped virus that is transmitted by arthropods. The virion of ASFV is formed by five concentric layers from the inside to the outside: the core, the inner core shell, the inner membrane, the capsid and the envelope. The core consists of the genome, the nucleoprotein, and the nucleolar organizer region. The inner core shell is mainly composed of the polyprotein pp62 and pp220 as a wide protein layer. The inner membrane is adjacent to the nuclear shell under electron microscopy and is a single lipid membrane containing p54, p17

and p12 proteins. The capsid is mainly composed of the p72 protein. The entry of ASFV into cells is a process dependent on temperature, energy, cholesterol and low pH value, and mainly infects mononuclear macrophages. Endocytosis mediated by clathrin and phagocytosis driven by actin are the main ways for ASFV to invade macrophages [1].

2.1. p22 protein

The p22 protein is encoded by the KP177L gene and is a major structural protein of ASFV. The p22 protein is an early protein during the formation process of ASFV and is located on the outside of the virus particles. It can be released from the surface of the virus particles by non-ionic detergents. The p54 and p30 proteins of ASFV expressed by baculovirus can induce the production of neutralizing antibodies in pigs, and some pigs can resist the attack of virulent strains. Therefore, expressing the p22 protein of ASFV by the baculovirus expression system and preparing monoclonal antibodies lay the foundation for the subsequent development of ASF subunit vaccines. The p22 protein plays an important role in the *in vitro* and *in vivo* replication of ASFV, and can simultaneously induce humoral immunity and cellular immunity in pigs [2].

2.2. p49 protein

The antigenicity of p49 protein was determined and new findings were obtained. The antigen index of p49 protein was 0.4524, indicating that p49 protein has certain antigenicity. The experiment also made certain predictions about p49 protein, suggesting that p49 protein is a hydrophilic unstable protein, containing 17 antigenic determinants, and β -turns and random coils account for a relatively large proportion of the total amino acids in the secondary structure. β -turns and random coils can protrude on the surface of the protein, have strong accessibility on the surface, and are prone to form antigenic epitopes. These indicate that p49 protein has immunogenicity, and there is a high possibility that there are potential advantageous antigenic epitopes, which can be used as a candidate protein for the development of ASFV vaccines [3].

2.3. p72 protein

The p72 protein is encoded by the B646L (VP72) gene, with a full-length gene sequence of approximately 1940 bp, located at the N-terminal of the viral genome [4]. It begins to be expressed in the late stage of viral replication. Many mutations have been made in the promoter of the late ASFV gene encoding the p72 capsid protein, including deletions, linker scanning substitutions, and point mutations. p72 is the capsid protein of ASFV and an important component of the icosahedral capsid of the virus particle. Three different subcapsomers p1, p2, and p3 located inside the capsid have been identified. These three proteins form a complex interconnecting network within the viral capsid, like glue, connecting adjacent capsomers and making the overall structure of the capsid more stable. In terms of distribution, p1 is located on the p72 trimer adjacent to the Trisymmetron and Pentasymmetron regions, and each p72 protein binds to one p1 protein [5].

2.4. CD2v protein

The CD2v protein is an outer membrane glycoprotein of ASFV, consisting of 402 amino acids. Its structure is similar to that of the CD2 adhesion receptor on the surface of T lymphocytes. Since there is a CD2 receptor on the surface of pig red blood cells, ASFV can easily be adsorbed onto the red blood cells and assist in the binding of red blood cells to infected cells, promoting the spread of the virus together with extracellular virus particles. The C-terminal of the CD2v protein helps the virus localize, promotes virus replication, and increases the spread of ASFV within the host. CD2v protein

can inhibit lymphocyte proliferation and participate in the replication and transmission of the virus and the immune escape mechanism. The analysis of the structure and function of the CD2v protein has promoted its application research and has become a hot topic in the current research on ASFV vaccines. Subunit vaccines, DNA vaccines, live attenuated vaccines, and virus vector vaccines targeting CD2v have been developed and have great development prospects [6].

3. Application of vaccines for ASF

3.1. Vaccine based on p72 protein

The p72 protein is the main capsid protein of ASFV. It plays a significant role in the viral infection process. During the stage of virus adsorption and invasion of host cells, p72 protein can recognize and bind to specific receptors on the surface of host cells such as porcine alveolar macrophages, helping the virus attach to the cell surface and creating conditions for subsequent invasion. p72 protein has a stable structure, which is conducive to maintaining the overall structural integrity of the virus particle and protecting the nucleic acid components inside the virus, enabling it to effectively complete the transmission and infection process in complex environments. Moreover, p72 protein has strong antigenicity and is an important target for serological detection. By detecting antibodies against p72 protein, it is possible to diagnose whether pigs are infected with ASFV.

3.2. Vaccines based on p30 and p54 proteins

A series of ELISA detection methods for ASFV can be established based on the characteristics of p54 protein. A blocking ELISA antibody detection method based on p54 protein has been established, and a competitive enzyme-linked immunosorbent assay (cELISA) based on p54 protein can also be made. The p54 protein can trigger a strong IgG immune response and can detect both IgG and IgM reactions. The detection method of ASF antibody using colloidal gold based on p54 protein is rapid and practical. The p30 protein and p54 protein have similar roles in the process of ASFV infection, and the two are often combined to develop subunit vaccines. They are also the target antigens of DNA vaccines. The protective rate of the ASF vaccine developed using p30 protein and p54 protein is 50%, which is higher than any vaccine prepared using p30 protein or p54 protein [6]. Existing research has proved that the antigenic epitope regions of p54 protein include: 23-29 aa, 36-45 aa, 65-75 aa, 72-94 aa, 93-113 aa, 114-120 aa, 118-127 aa, 137-150 aa, 175-185 aa. Epitopes are the key factors determining the antigenicity of viral structural proteins and inducing humoral immune responses. The study of epitopes is helpful to improve the detection efficiency of detection reagents and the development of subunit vaccines. This study identified a new antigenic epitope region of p54 protein 127-146 aa, which is expected to provide a valuable new target for improving the detection, monitoring and disease control of ASFV, and has potential application value in the monitoring and control of ASFV epidemics. It also provides a basis for the development of epitope vaccines targeting p54 [7].

3.3. The therapeutic effect of existing vaccines

A recombinant adenovirus Ad5-ASFV-P72 capable of expressing the ASFV P72 protein has been successfully obtained. CTL response may be a crucial factor for the body to exert protective effects. Therefore, developing a vaccine that can recognize CTL targets can stimulate a large number of CTL effector lymphocytes to eliminate cells infected by ASFV, and provide long-lasting immune protection. Recombining ASFV protective antigens into adenovirus or vaccinia virus vectors can achieve good cellular immunity and CTL responses. The ASFV p72 protein can be recognized by porcine CTL. The adenovirus was selected as the vector to express the ASFV p72 protein, and the

constructed recombinant adenovirus can be used in the subsequent development of ASFV adenovirus vector vaccines.

At present, adenovirus vectors have been widely applied in fields such as gene engineering vaccines, in vitro gene transduction and in vivo gene therapy. The human replication-deficient adenovirus vector Ad5 was used. It lacks the E1 region and E3 region responsible for encoding the early proteins of adenovirus, and the adenovirus lacking the E1 region cannot effectively replicate and produce various viral proteins. It must replicate in cells that can provide the function of the E1 gene (293A cells). This vector system also introduced the CMV promoter, enabling the exogenous gene to be highly expressed, and the expressed proteins can undergo post-translational modification and folding. Adenovirus vectors can carry multiple antigens and stimulate the body to produce strong humoral immunity or cellular immunity. Due to the ability of adenovirus vectors to infect respiratory and intestinal cells, they can be conveniently immunized through mucosal routes and induce mucosal and systemic immune responses. Adenovirus vectors can also be combined with other vectors for immunization [5].

Monoclonal antibodies designed based on p62 protein have been applied in the treatment of ASF. Currently, recombinant p62 protein of ASFV has been successfully obtained, and the monoclonal antibodies prepared from it are reactive with the virus. They have been preliminarily applied in immunohistochemistry, providing important biological materials for the establishment of ASFV immunological diagnostic methods and basic research on the structure and function of p62 protein.

Subunit vaccines are a new type of vaccine, containing only purified immunogenic antigens. These antigens are part of the pathogenic microorganism cells and specifically stimulate the immune system to produce specific antibodies. Due to the high expression level, low cost and high protective effect of subunit vaccines in disease treatment, they have received widespread attention in recent years. However, the immune effect of subunit vaccines is usually lower than that of traditional vaccines. It is necessary to enhance their immune response by adding adjuvants, optimizing delivery systems or binding antigens to particles. The p34 protein of ASFV is produced after hydrolysis and processing by polyprotein pp220, and it is the main component of the core shell of ASFV particles, with certain immunogenicity and protective effect. The efficacy of the prepared subunit vaccines is limited, so further enhancing the immunogenicity of the antigen is a problem that needs to be solved in research. Subunit vaccines have better safety than traditional vaccines. The correct selection of nanoparticle carriers is an important means to improve the immunogenicity of antigens [8].

Subunit vaccines are developed by using one or more specific proteins of ASF to trigger an immune response. This type of vaccine is relatively safe. It only contains partial components of the virus rather than the complete virus, and thus will not cause direct infection of pigs with ASF. However, its immunity is relatively weak. Specific adjuvants need to be added, the delivery system needs to be optimized, or the antigen needs to be combined with particles to enhance its immune response.

Ferritin nanoparticles can be used as carriers to modularly assemble and design the recombinant nanoparticles of the ASFV nuclear shell protein p34 through the SpyTag/SpyCatcher system. The aim is to prepare nanoparticles fused with antigens that can enhance the immune response in animals, providing new ideas for the preparation of subunit vaccines for ASF antigens. The specific antibody IgG (immunoglobulin) in the serum of mice was detected by indirect ELISA to observe the changes and titers. The results showed that the recombinant nanoparticle F-p34 group produced high-titer specific antibodies at 7 days and reached the highest level at 42 days, and the F-p34 group was significantly higher than the single antigen p34 group, the F group, and the PBS group (Figure 1).

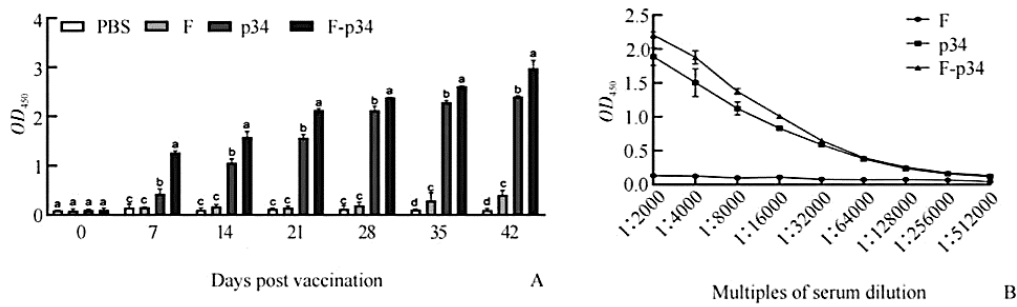


Figure 1: Detection serum antibody level in mice after immunization [8]. (A) and (B) The changes in specific antibodies in different time and serum dilution, respectively

Compared with the monomeric antigen p34, F-p34 elicited a stronger humoral immune response in mice. At 42 days after immunization, both F-p34 and p34 could produce high-titer specific antibodies with titers reaching above 1:256000, and the F-p34 immunization group showed higher levels than the p34 immunization group (Figure 1).

To determine the proliferation of lymphocytes in the lymph nodes after immunization, spleen lymphocytes from BALB/c mice 42 days after immunization were isolated and made into a 5×10^5 cells/mL cell suspension for inoculation into 96-well cell culture plates. The results showed that under the stimulation of ConA, p34 and F-p34, the SI values of spleen lymphocytes in the p34 and F-p34 immunization groups were significantly higher than those in the F and PBS immunization groups, and the SI value of lymphocytes in the F-p34 immunization group was significantly higher than that in the p34 group. The SI value of the RPMI-1640 group was 1, with no significant difference between groups. This indicates that compared with the monomeric antigen p34, the recombinant nanoparticle F-p34 more effectively stimulated the proliferation of spleen lymphocytes.

Ferritin was used as a nanocarrier to prepare a recombinant nanoparticle F-p34 targeting p34 protein. This enabled the p34 protein to be orderly displayed on the outer surface of ferritin nanoparticles, thereby increasing the loading and efficiency of the target protein p34. Compared with monomeric antigens, the recombinant nanoparticle F-p34 induced higher secretion of IL-4, IL-10, IFN- γ and IL-2, and the secretion levels were much higher than those of the control group, indicating that F-p34 not only induces humoral immune responses but also induces a strong cellular immune response [8].

For the combined use of subunit vaccines and inactivated virus vaccines, the combined immunization effect of the vaccines is jointly influenced by various factors such as the production factory of the vaccines, the antigen content in the vaccines, the immunization schedule, the level of maternal antibodies, the breed and management of pigs. The immunization effect varies greatly. Now, experiments have confirmed that when the two types of vaccines are mixed in a volume ratio of 2:1, the vaccine forms an oil-in-water-in-oil-in-water emulsion. It is not only uniform and stable, but also can effectively reduce the viscosity of the E2 subunit vaccine for swine fever and improve the needleability of injection [9].

4. Conclusion

This research has investigated various possible vaccines based on the studies of various proteins related to ASF. ASFV is the only known double-stranded DNA large envelope virus that is transmitted by arthropods. The entry of ASFV into cells is influenced by temperature, energy, cholesterol, and low pH values, and it mainly infects mononuclear macrophages. The p22 protein is an early protein formed during ASF and is located on the outside of the ASFV. There are studies that have expressed the p22 protein of the ASFV using the baculovirus expression system to prepare

monoclonal antibodies. The p22 protein plays a key role in the replication of the ASFV both *in vivo* and *in vitro*. The p49 protein has certain antigenicity. The p72 protein is a capsid protein of the ASFV and functions in the late stage of virus replication. The CD2v protein can inhibit lymphocyte proliferation and participate in the replication, transmission, and immune escape mechanisms of the virus. It has been found that there are now studies based on the p49 protein, p72 protein, and p54 protein to design vaccines. Subunit vaccines are currently the vaccines that are mainly valued by people because they are safe and only contain some components of the virus. There are now studies that have combined subunit vaccines and inactivated vaccines to effectively improve stability. Many studies have designed monoclonal antibodies based on specific proteins, such as monoclonal antibodies based on the p62 protein. These monoclonal antibodies have also laid the foundation for subsequent vaccine research. Adenovirus vectors have also been used in vaccine research and it has been found that good cellular immunity can be obtained. There are also experiments using nanoparticles as carriers to design vaccines and determine antigenicity, indicating that nanoparticles can improve the immune response.

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