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Review article

African swine fever virus: current state and future perspectives in vaccine and antiviral research

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ABSTRACT

African swine fever (ASF) is among the most significant of swine diseases for which no effective vaccines and antivirals are available. The disease, which is endemic in Africa, was introduced to Trans-Caucasian countries and the Russian Federation in 2007, where it remains prevalent today among domestic pigs and wild boars. Although some measures were implemented, ASF continues to pose a global risk for all countries, and thereby highlighting the importance of vaccine and antiviral research. In this review, an overview of research efforts toward the development of effective vaccines during the past decades is presented. As an alternative to vaccine development, the current state in antiviral research against ASFV is also presented. Finally, future perspectives in vaccine and antiviral research giving emphasis on some strategies that may allow researchers to develop effective countermeasures against ASF are discussed.

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1. Introduction

African swine fever (ASF) is a viral disease of wild and domestic pigs. African swine fever virus (ASFV), the causative agent of ASF, causes hemorrhagic fever in domestic pigs. Depending on ASFV virulence, clinical signs and infection course of ASF can vary significantly from long-term persistent infection to highly acute disease with 100% mortality. Upon infection with highly virulent strains, clinical signs include high fever, severe depression, anorexia, reddened skin, hemorrhagic lesions, cyanosis, and incoordination (Blome et al., 2013). Clinical signs are accompanied by severe thrombocytopenia and lymphopenia (Karalyan et al., 2012; Zakaryan et al., 2014). Animals usually die within 10–14 days of infection.

ASF circulates in pig populations on the African continent for many decades (Penrith et al., 2013). It has also affected domestic pigs in European countries such as Spain, Portugal, Italy and France. However, the disease was eradicated from Europe by the mid-1990s, except Sardinia, where it is endemic for many years (Sánchez-Vizcaíno et al., 2013). Although the ASF eradication programs in Sardinia are similar to programs conducted in Spain, Portugal and other EU countries, the occurrence of ASFV can be explained by the local socio-cultural, economic and productive factors impacting on the efficiency of eradication program (Martínez-López et al., 2015; Iglesias et al., 2015). In 2007, ASF was re-introduced onto the European continent via Trans-Caucasian countries, especially Georgia, from which it spread to

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Armenia, the Russian Federation, Belarus, Ukraine and most recently Poland and Lithuania (Sánchez-Vizcaíno et al., 2013; Gallardo et al., 2014; Woźniakowski et al., 2015). Due to the absence of vaccines and antiviral drugs, ASF poses a serious risk to all European countries.

Despite intensive research toward the development of ASFV vaccines, there is currently no effective vaccine that can prevent the global spread of ASF (Costard et al., 2009). The aim of this review is to present the current state in ASF vaccine development and antiviral research. Therefore, research efforts carried out over the past decades toward the development of effective vaccines are summarized. Moreover, other alternatives, such as antiviral compounds that are starting to gain relevance in light of the growing global threat of ASF are discussed. Finally, some perspectives in vaccine development and antiviral research are also highlighted.

1.1. Development of ASFV vaccines

Most viral vaccines were created by using two different strategies: viral inactivation and attenuation. Inactivated vaccines possess antigenicity but lack their ability to infect due to physical or chemical inactivation (Tlaxca et al., 2014). However, the major disadvantages of the inactivated vaccines are unsafety and incapability to generate prolonged immune response. In contrast, live-attenuated vaccines induce strong and lengthy immune response (Tlaxca et al., 2014). A number of strategies, such as targeted deletions, reverse genetics, recombination are now available for enhancing the effectiveness of live-attenuated vaccines (Plotkin, 2009).

Research to develop a safe and effective ASFV vaccine began in the mid-1960s, when investigators demonstrated that ASFV attenuated by passages in cell culture induced the production of swine antibodies. Pigs whose serum contained these antibodies failed to develop acute disease upon challenge with the homologues virulent ASFV strain (Stone et al., 1968). Forman et al. (1982) showed that pigs vaccinated with glutaraldehyde-fixed alveolar macrophages infected with ASFV and sonicated infected cells had an accelerated serological response after subsequent challenge with a virulent strain. Furthermore, pigs infected with non-haemadsorbing and non-fatal ASFV isolate demonstrated an increased resistance to the highly virulent ASFV/L60 isolate and survived infection with no major changes (Leitão et al., 2001). Interestingly, the immunization of pigs with non-virulent OURT88/3 isolate followed by a boost with a closely related virulent OURT88/1 isolate can induce the cross-protection against different isolates belonging to the same genotype I (King et al., 2011; Mulumba-Mfumu et al., 2015). Although this study showed that boosting with a virulent isolate did not ensure complete protection, this finding suggests that ASFV vaccines with cross-protection can be produced, thereby extending the vaccination strategies and their practical use (Dixon et al., 2013).

The latest study of Blome et al. (2014) demonstrated that inactivated ASFV preparations with modern adjuvants induced the production of ASFV-specific antibodies in pigs. Although all animals developed ASFV-specific antibodies, no protective effect of immunization was observed. This can be partly explained by the fact that ASFV-specific antibodies did not show a neutralizing activity in this study. However, a large body of evidence indicates that swine antibodies neutralize the infectivity of various virulent ASFV isolates *in vitro* and the loss of neutralizing activity depends on ASFV propagation in cell lines (Ruiz Gonzalvo et al., 1986; Zsak et al., 1993; Borca et al., 1994). Gómez-Puertas et al. (1997) confirmed that high- and low-passage ASFV differ in their susceptibility to neutralization.

Identification of antigens and their epitopes, which invoke a strong immune response, may help to develop effective vaccines against ASFV. Some research was done to identify such antigens. For instance, ASFV-specific cytotoxic T lymphocytes were shown to recognize and lyse the cells expressing the ASFV p30 and p72 proteins (Alonso et al., 1997; Leitão et al., 1998). Interestingly, antibodies to viral p30 protein inhibit ASFV internalization more than 95% in cells, whereas antibodies to p72, as well as to p54 are able to inhibit virus attachment, thereby revealing the role of these proteins in ASFV replication (Gómez-Puertas et al., 1996). Further studies of p32 and p54 showed that although the immunization of pigs with either recombinant p30 or p54 proteins induced the production of neutralizing antibodies, immunized pigs were not protected against acute ASF (Gómez-Puertas et al., 1998). In contrast, the combination of p30 and p54 proteins or a chimera of both these proteins may elicit partial protection against ASFV infection (Gómez-Puertas et al., 1998; Barderas et al., 2001). However, pigs immunized with baculovirus-expressed p30, p54, and p72 remained unprotected against ASFV (Neilan et al., 2004). These results indicate that antibodies to these proteins are not sufficient for complete immunity. Indeed, new evidence has highlighted the role of cytotoxic T lymphocytes that they can play in protection against ASFV. Oura et al. (2005) showed that pigs with depleted cytotoxic T lymphocytes were not protected from ASFV challenge. Furthermore, vaccination with DNA fusion plasmids and baculovirus based vectors designed to induce cytotoxic T lymphocytes response conferred partial protection against ASFV challenge, confirming the importance of cytotoxic T lymphocytes in vaccine development (Argilaguet et al., 2012, 2013). However, the above-mentioned attempts with inactivated, attenuated and subunit vaccines failed to confer total protection of experimentally infected pigs. Thus, future studies are still required for developing effective vaccines.

1.2. Development of antiviral agents

In the absence of a vaccine against ASFV, antiviral compounds developed at a reasonable cost may have very beneficial effects. Although it is always better to prevent disease rather than to treat, a possible application of antivirals could be to prolong host survival and to allow the infected pigs to generate a productive immune response against ASFV. Another option could be to apply antiviral treatment in areas located close to the affected farms in order to isolate the epidemic area and provide the designated authorities enough time to produce countermeasures.

Based on their inhibitory mechanisms, potential anti-ASFV compounds could be divided into two groups: (1) inhibitors that directly act on ASFV through its replication cycle, or (2) inhibitors that target host cell factors involved in virus replication. The replication cycle of ASFV consists of several steps including virus attachment, internalization, genome multiplication, assembly and release of the progeny virus. Specific agents that directly or indirectly block one of these steps may emerge as potential tools for the inhibition of ASFV infection.

Several agents affecting the early steps of ASFV infection were described. Sulfated polysaccharides were shown to affect ASFV attachment and subsequent replication. This was explained by negatively charged sulfate groups interacting with positively charged amino acids in the viral envelope, thereby inhibiting virus attachment to the cell (García-Villalón and Gil-Fernández, 1991). Macropynocitosis and/or clathrin-dependent endocytosis were reported to be the next steps in the ASFV entry (Hernaez and Alonso, 2010; Sánchez et al., 2012). Cholesterol in cellular membranes was shown to be relevant in this process (Bernardes et al., 1998; Quetglas et al., 2012). Thus, cholesterol removing agents like Methyl-β-Cyclodextrin were reported to inhibit viral replication (Hernaez and Alonso, 2010). Interestingly, amiloride, a potent inhibitor of macropinocytosis, caused a significant