REVIEW ARTICLE
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Problems of specific prevention of African swine fever

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This review presents the current state of the problem of development and application of the specific prevention of African swine fever (ASF) with a brief description of its etiology and pathogenesis. The unique nature of the ASF virus (ASFV) determines some limitations and the complexity of solving the problem of vaccine development. Such situation stimulated the development of highly specific diagnostic methods for rapid and accurate detection of the ASFV. In this regard, results of studies, including our own, concerning the comparative analysis of the genome of vaccine and virulent strains of the ASFV, as well as immunodiagnostic approaches to determine causes of high virulence and low protective activity of the ASFV, are briefly presented. Special attention is given to the issue related to the development of safe and effective vaccines against ASF. In this context disadvantages and possible advantages of live attenuated (LAV) and recombinant (RV) vaccines are considered in details. Results of recent studies on the assessment of the immunogenicity of genetically modified vaccines (GMV) which developed in various laboratories around the world are presented. The obtained data indicate that ASF vaccination is currently the most promising measure to stop the spread of this disease in our country and in the world, however, previous experience with ASF vaccination has revealed some problems in its development and application. The significant contribution of foreign researchers to the study of the basics of virulence of this pathogen and the study of its genes functions are noted. The possible further expansion of ASF in Europe and Asia in bordering Russia territories, as well as the established fact of the persistence of ASFV in wild boar population indicate a constant threat of its re-introduction into our country. In conclusion, the importance of developing a safe effective vaccine against ASF and the assessing of the possible risks of creating the artificial sources of the infection in nature as a result of its use is emphasized.

Keywords: *African swine fever, genome and virion structure; genetically modified virus; protective activity; antibody-dependent enhancement*

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Проблемы специфической профилактики африканской чумы свиней

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В обзоре представлено современное состояние проблемы разработки и применения средств специфической профилактики африканской чумы свиней (АЧС) с кратким описанием её этиологии и патогенеза. Понимание уникальности природы вируса АЧС определило ряд ограничений и сложность решения проблемы создания вакцины, что стимулировало разработку высокоспецифичных методов диагностики для быстрого и точного выявления возбудителя болезни. В связи с этим приводятся результаты исследований, включая собственные, касающиеся сравнительного анализа генома вакцинных и вирулентных штаммов вируса АЧС, а также иммунодиагностических подходов для определения причин высокой вирулентности и низкой протективной активности этого вируса. Особое внимание уделено вопросу, связанному с разработкой безопасных и эффективных вакцин против АЧС. При этом подробно рассматриваются недостатки и возможные преимущества живых аттенуированных (ЖАВ) и рекомбинантных (РВ) вакцин. Приводятся результаты последних исследований по оценке иммуногенности генетически модифицированных вакцин (ГМВ), созданных в различных лабораториях мира. Полученные данные свидетельствуют о том, что вакцинопрофилактика АЧС в настоящее время является наиболее перспективной мерой борьбы с распространением этой болезни в нашей стране и мире, однако предыдущий опыт вакцинации против АЧС выявил ряд проблем её разработки и применения. Отмечен значительный вклад зарубежных исследователей в изучение основ вирулентности этого возбудителя и функций его генов. Возможное дальнейшее распространение АЧС в странах Европы и Азии на приграничных с Россией территориях, а также установленный факт распространения вируса АЧС среди диких кабанов свидетельствуют о постоянной угрозе его повторной интродукции в нашу страну. В заключение подчеркнута важность разработки безопасной вакцины против АЧС и анализа рисков создания искусственных источников возбудителя в природе в результате её применения.

Ключевые слова: *африканская чума свиней; структура генома; структура вириона; генетически модифицированный вирус; протективная активность; антителозависимое усиление*

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Introduction

The evolutionary dynamics of viral populations, pathogens' high variability and ability to cross protective barriers of the susceptible host organism, introduction and spread of emerging infections to new areas of the world, anthropogenic impact on the epizootic situation demonstrate that studies on viral infections of animals, improvement of diagnostic techniques and prevention strategies are unfailingly significant [1–3]. The viral nature of the etiological agent of African swine fever (ASF) was discovered in 1921 by R.E. Montgomery who described it as "a highly contagious disease" with a near 100% mortality rate in affected animals and being different from classical swine fever [4]. Although initially the ASF virus was assigned to *Iridoviridae* by its virion morphology, the increasing knowledge of ASFV molecular biology led to its reclassification as the sole mem-

ber of a new DNA virus family, *Asfarviridae* [5]. Today, the ASF virus is assigned to the superfamily of large nucleocytoplasmic viruses (NCLDV) assumedly sharing a common ancestor [6]. The superfamily was offered to be renamed as *Megavirales*, capturing its members' structure and replication characteristics [6].

History of studies on the African swine fever virus

Throughout the entire history of ASF studies, researchers have encountered considerable difficulties trying to classify ASF virus isolates and creating prevention products; the difficulties are caused by the diversity of natural isolates of the pathogen, insufficient knowledge of its genome, complexity of its virion structure, and the small number of survived animals.

The contribution of Russian scientists to ASF studies deserves special attention: In 1961–1965, Ya.R. Kovalenko and the group of researchers of the All-Union Research Institute of Experimental Veterinary Medicine (VIEV) (M.A. Sidorov, L.G. Burba et al.) were first in Russia to conduct important experimental studies on ASF, having laid the foundation for further studies on exotic infectious diseases of animals. The obtained data made it possible to summarize the materials on biology of the virus, its antigenic properties, resistance to different physical and chemical factors, its persistence in the environment, clinical manifestation of the disease, and pathoanatomical changes [7]. The results of the studies conducted by the VIEV scientists served as the basis for the Interim Guidelines for Prevention and Control of African Swine Fever, which were approved in 1965 and incorporated ASF diagnostic and control methods.

The further studies by scientists of the Russian Research Institute of Veterinary Virology and Microbiology (RRIVVM of Russian Academy of Agricultural Sciences) in 1970–1980s (N.I. Mitin, Yu.I. Petrov, and others) resulted in development of the serological classification of natural virulent or attenuated isolates and strains of the ASF virus as well as in development of the first Russian vaccines based on attenuated virus strains [8–10]. Later, Balyshev et al. extended the serological classification: At present, isolates of the ASF virus are classified into 9 independent seroimmunological groups, while isolates whose serotype does not fit into the immunoassay results and new untyped virus isolates form a separate tenth group [11].

The long-lasting expansion of the ASF virus on the European continent in 1957–1995 provided ample data about the causative agent of the disease, pathogenesis and immunity against ASF. In the meantime, no effective and safe preventive ASF-specific strategy has been developed so far. The first attempts of mass vaccination failed due to high variability of the pathogen as well as due to lack of monitoring and recording of existing reservoirs and transmission vectors (wild boars and ticks) under field conditions, since after being vaccinated, the animals were repeatedly re-infected [12, 13].

Studies on the genome structure and antigenic properties of the African swine fever virus

The studies on various isolates of the ASF virus show that components of the population of its natural isolates are heterogeneous not only by their virulent properties and their ability to replicate in heterologous systems [14], but also by the serotype affiliation of individual variants [15]. Considering the serotype-specific immunity against ASF, the existence of more than 9 serotypes ASF virus makes development of vaccines extremely challenging.

In development of vaccines against ASF, the identification of the serotype of the circulating virus is a high-priority task. Unfortunately, to identify *in vitro* the serotype of a new isolate, researchers need a hyperimmune specific serum, which is difficult to obtain even using phosphonoacetic acid having a virustatic effect [16].

It should be noted that the ASF virus has a complex multilayer structure (**Fig.**) [17]. Its intracellular virions have an electron-dense nucleoid – a nucleoprotein core (70–100 nm in diameter) successively surrounded by two layers: internal lipid layer and middle layer – capsid composed of 1892–2172 capsomeres. The capsid exhibits icosahedral symmetry (T = 189–217) and is 172–191 nm in diameter. In their structure, extracellular virions have the third lipid-containing external layer (175–215 nm in diameter) [18]. With this structurally complex virion, the virus is highly resistant to environmental factors and host immune defense.

Intensive studies of the genome structure and antigenic properties of the ASF virus confirmed the assumption that its populations include immunologically and genetically different variants [15, 19]. The ASF virus genome encodes more than 160 various proteins [20] and its virion contains more than 50 structural proteins. Their molecular weight ranges from 10 to 150 kDa [21]; by their functional characteristics they are divided into 5 main groups: proteins involved in virus attachment and entry; proteins involved in virus morphogenesis; structural proteins; proteins associated with tropism and virulence of the virus; and regulatory proteins responsible for inhibition of apoptosis, synthesis of cellular proteins. They include humoral response inhibitors; proteins involved in interferon production; cytokine activators and chemokine inhibitors; modulators of the major histocompatibility complex; and modulators of cytotoxic T lymphocytes (CTLs, CD8+ T cells) and natural killer (NK) cells [22].

Consequently, proteins of the ASF virus play a significant role in modulation of the host immune response, thus adding to the factors hindering the development of vaccines against ASF. Regulatory proteins of the ASF virus suppress the production of interferon-β and interleukin-8; they enhance the production of anti-inflammatory cytokines and transforming growth factors [23]. Activated macrophages release cytokines and tumor necrosis factor (TNF-α) first inducing apoptosis of T cells and then B cells, thus causing the suppression of cellular and humoral branches of the immune response [24]. In addition to its ability to induce apoptosis, TNF-α increases vascular permeability, accelerates coagulation and, consequently, contributes to clot formation [25].

The ASF virus not only suppresses the host immune response, but also modifies the replication in host cells. The *J4R* protein expressed during late stages of the infection

cycle is present in the nucleus and cytoplasm of the infected cells and binds to the α-chain of the nascent polypeptide-associated complex (NAC) close to the cell membrane and in the cytoplasm. Assumedly, this viral protein inhibits the transcription of genes of the host cell [26].

Two variable regions of the ASF virus genome at 3' and 5' termini of the molecule contain multigene families (MGFs), which participate in regulation of gene expression and differ by the number of tandem repeats. Zsak et al. (2001) found that MGF 360 and 530 members play an important role in regulation of the tropism of the virus and are required for its effective replication in macrophages [27]. Virus mutants having a deletion of several MGF 360 and 530 genes caused early death of infected macrophages, thus proving the role of the virus-encoded proteins in regulation of apoptosis and, consequently, in cell survival [27].

Complex viruses such as herpes simplex virus, ASF virus or vaccinia virus have genes not only responsible for their replication, but also playing a major role in evading host immunological surveillance [23]. Finally, the ASF virus contains several genes that encode proteins sharing homology with host proteins, thus providing it with mimicry in respect of immune recognition [28].

Thus, due to its functional characteristics, the ASF pathogen not only effectively replicates in swine cells, but also alters their functions, inhibits the production of protective antibodies, and decreases the activity of T cells. Nevertheless, the data from D.L. Rock show that the vaccine against ASF can be created, as the protective immunity to the homologous virus has been proved; on the other hand, it is difficult to reach high levels of antibodies required for protection of animals against ASF, considering that the level of antibodies correlates with the level of protection against infection [29].

Challenges for development of vaccines against African swine fever

The current ASF panzootic gave an impetus to scientific research focused on development of an effective and safe vaccine against ASF, as the stamping-out strategy fails to demonstrate positive results in prevention of its spread. Many laboratories worldwide are working on vaccines in several promising avenues: live attenuated vaccines (LAV), genetically engineered vaccines (GEV) or marker vaccines (MV), subunit vaccines (SUV), and DNA vaccines.

Inactivated vaccines

As for inactivated vaccines (IV) against ASF, the studies have clearly demonstrated that the inactivated virus does not induce effective protection [30]. This

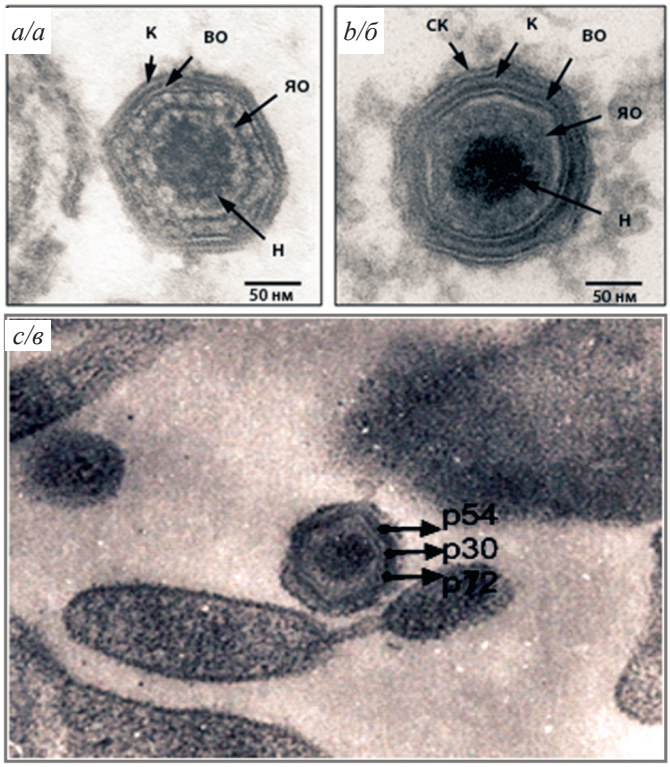

Fig. The structure of the ASF virus virion: *a* – formed intracellular virion; *b* – mature extracellular virion; *c* – localization of structural proteins in the virion [17].

СК – viral envelope; К – capsid; ВО – inner lipid envelope; ЯО (КО) – nuclear or core envelope; Н – nucleoid [18].

Рис. Структура вириона вируса АЧС: *а* – сформированный внутриклеточный вирион; *б* – зрелый внеклеточный вирион; *в* – локализация структурных протеинов в вирионе [17].

СК – суперкапсид; К – капсид; ВО – внутренняя липидная оболочка; ЯО (КО) – ядерная или коровая оболочка; Н – нуклеоид [18].

phenomenon is explained by the ASF virus uniqueness, when infected pigs do not develop virus-neutralizing antibodies (VNAbs) due to the multilayered structure of the virion: Lacking the supercapsid envelope, ASF virus retains its infectivity, using two alternative mechanisms to enter permissive cells: receptor-mediated endocytosis [17] and macropinocytosis [31]. As a consequence, the interaction of the virus with specific antibodies does not lead to neutralization of its infective activity.

The earlier studies on virus-neutralizing antibodies produced ambiguous results: Some researchers claimed the existence of passive protection induced by antibodies from surviving pigs. For example, Borca et al. (1994), Onisk et al. (1994) demonstrated that complete or partial protection could be provided through passive transfer of antibodies from recovered animals. In a number of cases, the presence of specific antibodies caused a decrease in viremia levels and increased the length of the incubation period [32, 33]. As shown by the data published by Escribano et al. (2013), the induction of specific antibodies provided different levels of protection, as ASFV-specific antibodies can induce protection, other than virus neutralization, using other mechanisms such as complement-dependent lysis, opsonization, and phagocytosis, antibody-dependent cell-mediated cytotoxicity [34]. On the other hand, the results of the experiments conducted by other researchers clearly demonstrated that passive transfer of antibodies did not provide any protection against ASF [35]. It has also been found that antibodies to some proteins of the ASF virus not only lack virus-neutralizing properties and provide no protection against the disease, but also can enhance the infection and hasten the death of infected animals. This phenomenon of antibody-dependent enhancement of infection has been thoroughly studied in other viruses replicating in cells of the immune system [36, 37]. Pershin et al. found that the administration of immunoglobulins from pigs recovered from ASF shortened the disease stage by 1–2 days and hastened the death of the immunized animals [38].

Through extensive studies, researchers have identified the main factors contributing to the absence of effective vaccines against ASF: The high level of variability of the ASF virus (the substitution rate in nucleotide sequences of ASF virus genomes was much higher compared to other large double-stranded viruses: the substitution rate in the ASF virus ranged from 1024 to 1025 and was comparable to the rate typical of RNA viruses, which usually have from 1022 to 1025 substitutions per site per year [39]; serotype-specific immunity (all the known isolates and strains of the ASF virus are classified into 9 serotypes); genetic and serotype-specific heterogeneity of the populations of some isolates; absence of virus neutralization by specific antibodies and existence of antibody-dependent enhancement typical of ASF.

Subunit vaccines

The thorough study of the immune response to ASF using recombinant proteins, which was performed by Gómez-Puertas et al. (1998), demonstrated that antibodies to such proteins of the ASF virus as p30 and p54 were required for protective immunity [40], while the transfer of additional antibodies against p72 suppresses it. Moreover, it was observed that the immunization with recombinant p72 resulted in early death of animals infected with the ASF virus (after 1.5–2 days) and more pronounced clinical symptoms compared to the control groups. These studies served as the basis for development of subunit vaccines against ASF, as effective and safe vaccines should be created with consideration for the phenomenon of antibody-dependent enhancement; then the emphasis should be placed on development of a highly immunogenic vaccine that change the antibody formation towards protective antigens to avoid infection enhancement and virus masking with antibodies against non-protective proteins. Nevertheless, currently, the development of subunit vaccines is impeded by the absence of identified key antigens of the ASF virus, which participate in induction of immunity mediated by T cells; the number of tested proteins is very small:

– immunization of pigs with recombinant p30 and p54 delayed the onset of the disease and viremia, though 50% of the pigs survived for > 45 days [40];

– as mentioned previously, immunization with recombinant proteins p54, p30, and p72 delayed the onset of the fever, but did not change the time of death [41].

Similarly to p30 and p54, immunization with CD2v also provided partial protection against infection with the virulent strain. Recent studies provided the evidence that CD2v proteins and (or) C-type lectins were important for protection against homologous infection with the ASF virus [42]: When immunized with recombinant CD2v proteins, three pigs were completely protected, in one pig no viremia was detected; in two pigs, it was decreased 10–100 times. At the moment, the group of researchers led by L.K. Dixon is actively looking for protective proteins for the ASF virus. Goatley et al. (2020) described the induction of ASFV-specific antibodies in response to immunization with different pools of recombinant proteins. The researchers found a composition of 8 proteins, which provided animals with 100% protection against the challenge infection with the virulent strain of the ASF virus [43]. However, there are only preliminary data on the major protective proteins of the ASF virus as potential components of the subunit candidate vaccine.

Live attenuated vaccines

Currently, one of the promising approaches is development of live attenuated vaccines. The studies performed in different research laboratories focusing on development of specific preventive means against ASF have shown that the immunization with the attenuated ASF virus protects against infection with closely related virulent isolates, i.e. virulent isolates of the respective serotype [13, 30, 44]. The analysis of the immune response induced by the attenuated variant of the ASF virus confirmed the presence of protective response against infection with the homologous virulent virus [45]. It has been found that the vaccination of pigs with the naturally attenuated strain OURT 88/3 protects them against infection with homologous virulent strains of the ASF virus [45], though it al-

so provides partial cross protection against heterologous virus strains. The protection level in the animals ranged from 66% to 100% depending on the body weight and age of the pigs as well as on the viral dose used for the challenge infection and on routes of its administration. At the same time, pigs immunized against one serotype of the virus had severe clinical and pathological ASF manifestations, ending in their death, when they were infected with the virulent virus of another serotype, though 10–30% of the immunized pigs had cross protection against the heterologous ASF virus [10].

The analysis of the data from studies on resistance to ASF, which were conducted by researchers from different countries, led to the conclusion that the main role in the protective immune response in pigs belonged to cell immunity mediated by CTLs, which inhibit the replication of the virus in infected cells [46]. Not surprisingly, the replication of the ASF virus, first of all, disrupts the activity of this sector of the immune response. In animals immunized with vaccines based on attenuated strains, specific antibodies and activated CTLs play a significant role in protection development. Oura et al. (2004) demonstrated that depletion of CTLs decreased or completely disrupted the protection established by immunization with the attenuated strain OURT 88/3 [47]. Meanwhile, the intensive research conducted in the recent years has shown that the presence of specific antibodies and activated CTLs is by no means the only condition of animals' resistance to challenge infection. The early apoptosis of the infected cells also disrupts the replication of the ASF virus; therefore, the activity of virus inhibitors should be blocked. After pigs had been immunized with the attenuated virus, the cross protection resulting from their infection with virulent isolates of different genotypes correlated with its ability to stimulate effectively the production of interferon γ by lymphocytes in the immunized animals [48].

However, attenuated vaccines can frequently cause side effects: From 2% to 30% of the vaccinated pigs developed complications after the vaccination, including intermittent fever and viremia, rhinitis, pneumonia, locomotor disorders, necrotic lesions, abortion, and even death of test animals. It means that three main questions regarding the development of live attenuated strains – candidates for vaccines against ASF – remain unsolved:

1) the potential of animals' resistance and virus transmission under field conditions has not been studied;

2) the safety-related problems: The immunized animals have such side effects as intermittent fever, cardiorespiratory disorders, hemorrhagic skin lesions, and musculoskeletal disorders;

3) live attenuated vaccines bring quick results, but it will take several years to assess their safety under field conditions.

Genetically engineered vaccines

The further studies on ASF preventive vaccines revealed the immunosuppression of the monocyte-macrophage system, which made the vaccine development even more challenging [49]. Identification of the genes responsible for replication of the virus in specific cell cultures, in ticks and pigs is essential for making targeted changes in these genes and for using genetic engineering manipulations to obtain attenuated strains free of the drawbacks typical of naturally and laboratory-attenuated strains.

For this reason, ASF vaccine developers focus their efforts on live GEVs using targeted deletion of genes. This approach makes it possible to differentiate infected animals from vaccinated ones (Differentiating Infected from Vaccinated Animals, the DIVA-strategy). Vaccination using the genetically modified ASF virus obtained through inactivation of specific genes responsible for virulence or immunosuppression significantly increases safety of vaccines: ASF GEV with deletions in thymidine kinase genes *9GL* (*B119L*), *DP71L* in MGF 360/505 – induced the protective immune response against infection with the homologous virulent isolate [50, 51]. In 2020, Borca et al. published their data demonstrating that the deletion of the *I177L* gene (ASFV-G-ΔI177L) caused the complete loss of virulence of the original variant of the virus. As a result, after the experimental infection with the original highly virulent Georgia 2007/01 virus, all the 20 animals vaccinated with ASF GEV survived [52]. Although this approach produced successful results, there is still no information about the stability of the vaccine virus, its possible reversion to the virulent type, the duration of immunity and many other important parameters. The authors of this study filed patents in 2016–2017 for other ASF GEV variants, which also prevented death of animals after the challenge infection: ASF GEV based on deletion of MGF genes and created through deletion of MGF 360 genes: *12L*, *13,* and *14L* from the original isolate Georgia 2007/01; MGF505: *1R*, *2R,* and *3R* responsible for virulence; Δ9GL-UK ASF GEV based on the Georgia 2007/01 isolate, created by deleting virulence-associated *9GL* (*B119L*) and *UK* (*DP96R*) genes and protecting against the infection with the Georgia 2007/01 isolate; ASFV-G GEV based on the Georgia 2007/01 isolate, developed by deleting the *9GL* (*B119L*) gene fragment and providing protection against infection with homologous Georgia 2007/01 isolate.

However, in some cases immunization with deletion mutant viruses fails to produce a protective effect. For example, the animals immunized with ASF GEV with deleted MGF 360 and 505 genes and *9GL* gene of the Georgia 2007/1 virus did not acquire resistance to infection with the original virus [53]. The vaccination with the modified virus obtained by deleting the *9GL* gene from the genome of the virulent Georgia 2007/1 isolate did not produce a protective effect after the challenge infection with the original strain. The experimental deletion of two *9GL* and *UK* genes demonstrated an increased protective effect only compared to the deletion of the *9GL* gene [53].

DNA vaccines

The United States is not the only country trying to develop effective vaccines for preventive vaccination against ASF. The research and development in this area are conducted in China (Zhejiang Hailong Biotechnolo-

gy Co., Ltd), Spain (UCM – the OIE reference laboratory), and Russia (the Kazan State Academy of Veterinary Medicine). In Spain, a candidate vaccine was developed from the non-hemadsorbing (the property directly related to virulence) genotype II Lv17/WB/Rie1 ASF virus isolated from a wild boar in Latvia in 2017. The genome of this isolate contains a mutant gene encoding the truncated version of the CD2v-like protein responsible for hemadsorbing properties of the virus. The oral vaccination of wild boars with the candidate vaccine provided 92% protection against infection with the highly virulent ASF virus Arm07 isolate (1 boar of 12 died). The authors are conducting studies on resistance, reversibility, and biological properties of this isolate [54]. The recent study on the CD2v deletion mutant of the highly virulent ASF virus BA71 isolate has demonstrated that it is possible to produce protective immunity against infection both with the homologous and with the heterologous ASF virus [55].

The earlier approaches to development DNA-vaccine-based protection against ASF also demonstrated ambiguous results. For example, the immunization with pools of DNA encoding proteins of the ASF virus provided 30–50% protection (Argilaguet et al. (2012)). The immunization with plasmids containing genes of ubiquitinated *CD2v*, *p30*, and *p54* proteins produced a pronounced CTL-response and provided partial protection while the production of specific antibodies was absent [56]. It has been found that DNA-based vaccines and vaccines based on attenuated viruses induce cellular and humoral specific immune response against the ASF virus, though they have provided only partial protection against infection so far [56]. Lokhandwala et al. (2016) achieved a robust cellular and humoral immune response after using the immunization with the recombinant adenovirus producing specific proteins of the ASF virus and the re-immunization with the recombinant modified vaccinia virus Ankara (MVA) carrying the genes identical to the genes of the ASF virus. However, these experiments were not completed by challenge infection of the immunized animals, which did not allow establishing a positive result of immunization [57].

The possibility of achieving the protective immunity against ASF was confirmed by studies on DNA immunization, which demonstrated the correlation between the development of protection against lethal infection with the ASF virus and the production of a large number of antigen-specific CTLs induced by the DNA vaccine [58]. As can be seen from the published data, all the above variants of vaccines are candidate vaccines and require further studies before they can be used in agriculture.

Thus, the absence of effective and safe vaccines against ASF is explained not only by the structural uniqueness of the ASF virus, the large number of proteins involved in suppression of the host immune response, and the high variability of the virus, but also by the need to fine-tune the pathogen research and modification methods, to develop cell culture techniques for vaccine variants, and by the time required for creating optimum conditions for production of virus-containing materials or recombinant antigens. The prototype vaccine will have to go through multiple stages, including planning of its commercial manufacturing, evaluation of its safety, development of approaches to its further use for prevention of the ASF virus spread. As the compliance with the OIE standards and the DIVA-strategy requires the vaccines that make it possible to differentiate between vaccinated and infected animals, the respective testing systems must be developed and approved to differentiate vaccinated animals from naturally infected or recovered animals.

Conclusion

Concluding the review, we would like to note that ASF is not the only infection that causes difficulties to scientists as vaccines do not guarantee effective protection against the disease due to the specific characteristics of the pathogen and its effect on the immune system: porcine reproductive and respiratory syndrome, chlamydia infection, leukemia, and some other diseases of viral or bacterial etiology cannot always be conquered using preventive vaccination [59].

Therefore, development of effective and safe vaccines against ASF is a long process, which involves close cooperation of researchers, veterinary specialists, government authorities, and intergovernmental agencies authorizing clinical trials and use of vaccines, focusing on higher levels of biosafety of pig farms.

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