Reviews, challenges

IMMUNOBIOLOGICAL AND MOLECULAR GENETIC PROPERTIES OF NON-HEMADSORBING AFRICAN SWINE FEVER VIRUS STRAINS
(review)

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A b s t r a c t

African swine fever virus (ASF, *Asfivirus*, *Asfarviridae*) is the most serious problem for the swine industry worldwide. The proposed review presents the results of the study of non-hemadsorbing strains of the African swine fever virus (ASF, African swine fever virus). According to published data, most of the non-hemadsorbing strains of the ASF virus isolated in nature or obtained in laboratory conditions are weak or avirulent and have the property of forming immunological protection against homologous virulent hemadsorbing isolates or strains in subsequent infection of pigs (J.D. Vigário et al., 1970). On the African continent, avirulent non-hemadsorbent strains of ASF virus were usually isolated from persistently infected warthogs (*Phacochoerus* spp.), bush pigs (*Potomochorus porcus*) and soft mites *Ornithodoros moubata* (A. Pini, 1976; G.R. Thomson et al., 1979). In Europe (Portugal, Spain) and Asia (China) − from persistently infected domestic pigs (*Sus scrofa domesticus*), wild boars (*Sus scrofa*) and from *Ornithodoros erraticus* (*marocanus*) ticks (F.S. Boinas et al., 2004; C. Gallardo et al., 2019; Sun E. et al., 2021). The review focuses on the use of non-hemadsorbing strains in order to study immunological mechanisms of protection against ASF. In experiments with the OURT88/3 strain, CD8+ T-cells were shown to have an important role in immunological protection against ASF. The cross-protection induced by the OURT88/3 strain against infection with virulent isolates of unrelated genotypes correlated with the ability of these isolates to specifically stimulate the production of IFNγ by lymphocytes of the immunized pigs (C.C. Abrams et al., 2013). Experiments with the non-hemadsorbing strain NH/P68 demonstrated that a high levels of specific antibodies to the ASF virus is characteristic to the chronic form of the disease, while low levels of antibodies were noted in asymptomatic pigs after intranasal and intramuscular immunization (A. Leitão et al., 2001; C. Gallardo et al., 2019). The low pathogenicity of non-hemadsorbing isolates is associated with the loss of virulence factors due to large deletions close to the left end of the genome or smaller deletions and substitutions in genes encoding virulence factors elsewhere in the genome (F.S. Boinas et al., 2004). The loss of the hemadsorbing properties of the ASF virus is associated with deletions and/or a shift in the reading frame in the *EP402R* gene (R.J. Rowlands et al., 2009; R. Portugal et al., 2015; K.A. Mima et al., 2015). In terms of possible practical application of non-hemadsorbing strains this paper presents results on reducing adverse clinical reactions in pigs inoculated with deletion mutants of strains OURT88/3 and NH/P68 (M.L. Nogal et al., 2001; C. Hurtado et al., 2004; A.G. Granja et al., 2009). Naturally attenuated non-hemadsorbent strains of the ASF virus are used in research focused on the creation of candidate live vaccines. In experiments conducted with their use, up to a 100 % protection against homologous virulent isolates and strains of ASF virus was obtained in domestic pigs (K. King et al., 2011; P.J. Sánchez-Cordyn et al., 2017; C. Gallardo et al., 2018; C. Gallardo et al., 2019; P.J. Sanchez-Cordon et al., 2020) and wild boars (J.A. Barasona et al., 2019).

Keywords: African swine fever, non-hemadsorbing isolates, non-hemadsorbing strains, candidate vaccines

African swine fever (ASF) is a cruel infectious diseases of pigs and wild boars. ASF is currently the most serious problem for the pig industry worldwide.

Due to the lack of commercial vaccines [1-3], the only way to combat ASF remains the total destruction of domestic pigs and wild boars in the foci of infection [4, 5]. Due to the severe economic impact on the international trade in pigs and pork products, ASF is on the list of notifiable diseases.

The disease is caused by a single member of the *Asfarviridae* family (*Asfar*, *African swine fever virus*), a large DNA-containing virus that infects domestic and wild pigs (*Suidae*) [6-8]. In southeastern and southern Africa, ASF is maintained in a sylvatic transmission cycle between warthogs (*Phacochoerus* spp.), bush pigs (*Potomochorus porcus*) and soft mites (*Ornithodoros moubata*) [9]. Typically, in natural hosts, including ticks, ASFV causes a subclinical chronic or inapparent form of infection [10, 11]. Isolates and strains of the ASF virus differ in pathogenic, antigenic, haemadsorbing, genetic properties [12-14]. In particular, based on the 3'-terminal sequences of the B646L gene encoding the main p72 capsid protein, 24 ASFV genotypes have been identified in Africa [15-17]. ASF genotype I virus caused outbreaks of ASF outside the African continent from 1957 to 1991 in Portugal, Spain, France, the Netherlands, on the island. Madeira, in Italy, Cuba, Malta, about. Sardinia (Italy), in Brazil, the Dominican Republic, Haiti and the USSR [18-20]. All of them were eliminated (with the exception of the outbreak on the island of Sardinia). In 2007, the ASF virus of genotype II was introduced from Africa to Georgia, which received the name Georgia 2001/1 strain, the derivatives of which spread to other countries of the Caucasus and Europe [21-23]. The general picture of the development of the incidence indicates that ASF has become epizootic with the involvement of populations of both domestic and wild pigs [24-26]. In 2018, Georgia 2001/1-like ASFV genotype II was isolated in China and spread to 15 other Asian countries within three years [27-29]. However, along with the emergence of low-virulence genotype II isolates, two strains of genotype I ASF virus were isolated from diseased domestic pigs in China in mid-2021 [30-31]. Special attention of specialists was caused by the fact that both strains were characterized as non-hemadsorbing [31].

This review analyzes studies of non-hemadsorbing strains of the ASF virus, considers the features of their immunobiological and molecular genetic properties, and their use in fundamental and applied scientific research. In a number of works, when describing the viruses under study, the term "isolate" is more often used. In the context of this article, we mainly used the term "strain", based on the fact that a strain is a local population of a virus identified by modern classification tests with original, stable properties (features), and an isolate is a virus isolated from a specific source.

Immunobiological properties of non-hemadsorbing strains of the ASF virus. The vast majority of ASF virus isolates are characterized as virulent and haemadsorbing. In 1968, L. Coggins reported the isolation of non-haemadsorbing subpopulations of the ASF virus [32]. Further studies have shown that the loss of the ability to induce hemadsorption for the ASF virus during reproduction in cell culture is a common phenomenon [33-35]. A number of researchers have noted that non-hemadsorbing strains of the ASF virus isolated in nature or obtained under laboratory conditions have low virulence and the ability to form immune protection against subsequent infection of pigs with homologous virulent hemadsorbing isolates [36, 37]. On the African continent, low-virulence or avirulent non-hemadsorbing isolates of the ASF virus, as a rule, were isolated from persistently infected warthogs, bush and domestic pigs, ticks of the genus *Ornithodoros* [38-40]. According to the EU Commission, 1% of samples obtained from domestic pigs in the Iberian Peninsula between 1968 and 1976 contained non-haemadsorbing ASF viruses [41]. Researchers who have attenuated ASFV in the laboratory to obtain candidate live vaccines have empirically arrived at a

method for selecting avirulent strains based on their reduced ability to induce haemadsorption during in vitro reproduction [42]. It should be noted that virulent non-hemadsorbing isolates have sometimes been isolated in nature. For example, of the two non-hemadsorbing isolates tested, one (Lillie-148) was virulent, while the other (Zaire) caused disease and death in only 33% of pigs [40].

The first naturally occurring non-hemadsorbing isolates of ASF virus were obtained from pigs in southern Portugal, where most of the pig herds were sero-positive [43, 44]. This was preceded by outbreaks of ASF in the Iberian Peninsula in 1957 and 1960, which served as the beginning of an epizootic that lasted until the 1990s and still persists on the island. Sardinia in Italy. From the virulent haemadsorbing strain Lisbon 60 isolated during the second outbreak, a haemadsorbing "vaccine" strain 1455 was obtained as a result of 150 passages in primary culture of pig bone marrow cells. vaccinated animals, 7% of pigs developed unacceptable post-vaccination reactions, including pneumonia, movement disorders, skin ulcers, abortions and death of animals [45, 46]. More recently, a non-pathogenic, non-hemadsorbing strain of ASF virus NH/P68 (NHV, NHA2) was isolated from a pig with chronic ASF in Portugal. Also in Spain, 206 non-hemadsorbing isolates were obtained between 1965 and 1974 (47). It was noted that it was more difficult to isolate non-hemadsorbing ASFV isolates than hemadsorbing isolates, since the viremia caused by them was sporadic, and the virus accumulated in the organs of pigs in small quantities. Experiments have shown that non-pathogenic non-hemadsorbing isolates were less efficiently transmitted via contacts than virulent hemadsorbing isolates (40-50% vs. 100%). Infection of pigs with non-pathogenic non-hemadsorbing isolates could be the reason for the seropositivity of some herds in the absence of clinical symptoms in pigs. Based on the fact that inoculation of the NH/P68 strain resulted in protection against subsequent lethal challenge with the virulent Lisbon 60 strain, it was concluded that they are antigenic related [48].

Twenty years later, two types of ASF virus isolates of different pathogenicity were isolated from *O. erraticus* (*marocanus*) ticks collected from pigs and piggeries in southern Portugal. Isolates of the first type caused 100% death of pigs from the acute form of ASF, the second, in particular OURT88/3, did not cause clinical signs of disease and death of animals, although antibodies against the ASF virus were detected in all infected pigs [49]. It is believed that the direct ancestor of the OURT88/3 strain was the NH/P68 strain, which also replicates in ticks and is well adapted to the natural transmission cycle [50]. Two assumptions have been put forward regarding the origin of non-pathogenic non-hemadsorbing isolates in the Iberian Peninsula: they were obtained either from an attenuated vaccine strain or from an initial virulent isolate capable of persisting in the domestic pig—tick—wild boar cycle. Pigs inoculated with the non-haemadsorbing strain OURT88/3 were protected from death after infection with the related pathogenic haemadsorbing strain OURT88/1. Less effective protection was achieved when recovered pigs were injected with more distantly related isolates or strains of the ASF virus. It has been noted that after infection of immune pigs with the Lisbon 57 strain isolated during the first outbreak of ASF in Portugal in 1957 and the African isolate Malawi LIL20/1, the animals died, although the onset of clinical manifestation of the disease was delayed in time [51-53].

A comparison was made between the intramuscular and intranasal routes of immunization of pigs with different doses of the non-haemadsorbing ASF virus strain OURT88/3 [54]. With intranasal administration, two clinical groups were formed: pigs that developed intermittent clinical manifestations (10^3 and 10^4 $TCID_{50}$, 100% protection against OURT88/1), and animals that developed

chronic ASF (a dosage of 10^5 TCID$_{50}$, 66% protection. Pigs immunized intramuscularly with low and medium doses (10^3 and 10^4 TCID$_{50}$) showed a lower percentage of protection (50 and 66%). In blood samples throughout the study period, a low content of the virus genome was found. Interestingly, intramuscular immunization did not result in signs of chronic ASF in protected pigs. Viremia was not detected as early as 7 days after virus inoculation. These results indicated that the route of administration and the dose of virus determined the outcome of immunization with the naturally attenuated OURT88/3 strain. In studies with the low virulent NH/P68 strain, intranasal immunization also induced higher protection than intramuscular immunization [53]. A correlation was established between late viraemia after NH/P68 immunization (14 days after virus inoculation) and the appearance of pigs with chronic ASF. This ratio was not observed in protected pigs immunized intranasally with OURT88/3 strain, where late viremia has been described in animals without chronic ASF [48, 55].

It has been established that the OURT88/3 strain induces a high degree of protection against lethal infection by related virulent isolates of the ASF virus [49, 56, 57]. Experimental immunization of pigs with OURT88/3 followed by challenge with the closely related virulent strain OURT88/1 induced protective immunity in European domestic pigs against challenge with two virulent African isolates of ASFV: Benin 97/1 genotype I from West Africa (85.7%) and Uganda 1965 genotype X from East Africa (100%). More than 78% of pigs infected with Benin 97/1 and 50% infected with Uganda 1965 showed no signs of disease or development of viremia [57].

In the Democratic Republic of the Congo, the non-hemadsorbing strain Mfuati-79 (immunotype II, genotype I) was isolated from domestic pigs in 1979. Fifteen days after intramuscular immunization with the Mfuati-79 strain at $10^{3.0}$-$10^{4.0}$ TCD$_{50}$, pigs developed resistance to intramuscular infection with the homologous virulent haemadsorbing strain Congo-49 (immunotype II, genotype I) at $10^{5.5}$-$10^{7.5}$ HAU$_{50}$ [58].

In 2017, a non-hemadsorbing ASF virus isolate Lv/17/WB/Rie1 genotype II was isolated in Latvia (59). In pigs inoculated intramuscularly with the Lv/17/WB/Rie1 isolate, an asymptomatic form of infection was observed, periodic and weak viremia was manifested, and a high content of virus-specific antibodies was noted in blood sera. In addition, 2 months after primary infection with the Lv17/WB/Rie1 isolate, two pigs infected with the virulent hemadsorbing Latvian isolate survived. Despite the fact that the number of animals was small, these results, according to the authors, open the prospect of using the Lv17/WB/Rie1 isolate as an object for the development of live attenuated vaccines, as is the case with the NH/P68 and OURT88/3 strains. Importantly, the study illustrates the natural evolution of the ASF virus, including the emergence of less avirulent non-haemadsorbing isolates over time in the absence of *Ornithodoros* ticks.

For the first time in 14 years of global ASF panzootic caused by strains similar to Georgia 2007/1 genotype II, outside of Africa and about. Sardinia in Henan and Shandong provinces of the PRC isolated two strains of genotype I (HeN/ZZ-P1/21 and SD/DY-I/21) from domestic pigs, which were non-hemadsorbing and caused chronic ASF disease [31]. Phylogenetic analysis showed some differences between the strains HeN/ZZ-P1/21 and SD/DY-I/21 and their commonality with the Portuguese strains NH/P68 and OURT88/3.

It has been known for many years that pigs that recover from infection with ASF virus can be protected from disease and/or death when subsequently infected with related virulent isolates of the virus [60-62]. In addition, pigs inoculated with naturally attenuated or laboratory-selected cell culture passaged ASF viruses may also be protected from challenge by homologous virulent isolates [49,

63]. The non-hemadsorbing strains NH/P68 and OURT88/3 of the ASF virus are effectively used in the study of the mechanisms of formation of immune defense and to determine the significance of various genes in the pathogenicity of the virus. In experiments with the OURT88/3 strain, an important role of CD8+ T cells in the immune defense against ASF was established. Monoclonal antibody depletion of this cell subpopulation abolished the protection induced by the OURT88/3 strain against infection with the virulent OURT88/1 isolate [56]. The possible role of antibodies in protection against ASF has been shown in experiments on the passive transfer of antibodies from immunized to intact pigs [64-66]. It has been found that neutralizing antibodies are not effective enough, but other antibody-mediated protective functions are possible, in particular antibody-dependent cytotoxicity [64, 67].

Experiments with the NH/P68 strain have shown that a high level of specific antibodies to the ASF virus is characteristic of the chronic form of the disease, while a low level is noted in asymptomatic pigs after intranasal and intramuscular immunization [46, 57]. Regardless of doses and methods of immunization with strain OURT88/3, high levels of antibodies against the structural protein p72 were observed in pigs both with signs of chronic ASF and without them, and even in 50% of immunized pigs that were not protected from infection with a homologous virulent isolate [68]. Cross-protection induced by strain OURT88/3 against infection with virulent isolates of ASF virus from unrelated genotypes correlated with the ability of these isolates to specifically stimulate the production of IFNγ by lymphocytes of immunized pigs [57, 69].

Studies measuring the activity of NK cells in pigs inoculated with the NH/P68 strain revealed the functional role of this subset of lymphocytes in antiviral defense. In animals that remained healthy after the introduction of the NH/P68 strain and became resistant to infection with the Lisbon 60 strain, an increased number of NK cells was noted on the 7th day after inoculation. In some pigs, high NK cell activity was observed throughout the experiment. In contrast, in animals that developed chronic ASF after inoculation with NH/P68, NK cell activity was similar to or slightly higher than that of control animals. Virulent ASF virus isolates suppressed NK cell activity in pigs [70]. In vitro NK activity of porcine mononuclear cells was suppressed by both low- and high-virulence ASF virus isolates [71]. These data support the notion that immunity to ASFV depends, at least in part, on cellular mechanisms, in particular NK cells [48].

Structural, functional, comparative genomics of non-hemadsorbing ASF virus strains. In the ASF defense strategy, the use of a live attenuated vaccine is considered to be preferable because it elicits immune responses against all viral antigens that the host normally encounters during infection [72, 73]. With this in mind, studies mainly use naturally attenuated non-hemadsorbing strains OURT88/3 and NH/P68. Including the possibility of reducing adverse clinical reactions in pigs inoculated with deletion mutants of strains OURT88/3 and NH/P68 while maintaining high protection against homologous virulent isolates of the ASF virus [74-76].
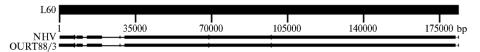
It is known that the deletion of the DP96R gene in the DNA of the virulent E70 strain did not affect the growth characteristics of the ASF virus in macrophage cell cultures in vitro, but the degree of viremia in pigs inoculated with a deletion mutant of the virus was reduced 100-1000-fold [77, 78]. Similar deletions of the *DP71L* and *DP96R* genes from the DNA of the OURT88/3 strain also did not reduce the replication of the deletion mutant OURT88/3ΔDP2 in primary porcine macrophages in vitro compared to the parent strain OURT88/3. However, two of the six pigs inoculated with the deletion OURT88/3ΔDP2 virus were not protected from subsequent challenge with the virulent OURT88/1 strain, while all

six pigs inoculated with the parent strain OURT88/3 were protected (69). Deletion of the *A224L* gene encoding an apoptosis inhibitor from the genome of strain NH/P68 did not affect the ability of the deletion mutant NH/P68DA224L to protect pigs from the homologous virulent Lisbon 60 strain. one pig immunized with NH/P68DA224L). In contrast, the parental NH/P68 virus completely protected the animals after infection with Arm07. Not only did the pigs not show any noticeable clinical signs after infection with Arm07, but no virus was found in their blood or tissues. Interestingly, from pigs immunized with the parent non-hemadsorptive strain NH/P68, the virus was effectively transmitted to controls within 3-4 weeks after initial infection. However, transmission of the virulent Arm07 virus to control pigs was not observed [72].

The question is obvious: what is the difference between the genomes of virulent hemadsorbing and attenuated non-hemadsorbing strains of the ASF virus? The genomes of two non-hemadscoping strains of the ASF virus, NH/P68 and OURT88/3, have been well studied. In the first one, the genome consists of 172051 bp, in the second, of 171719 bp. 158 open reading frames (ORFs) are encoded in their genomes, the similarity is 99.98% [69, 79].

Variants of the ASF virus genomes are mostly the result of the presence of a different number of genes of multigene families (MGF) on the left and right variable regions (LVR and RVR). MGFs are specific to the ASF virus and have no obvious homology with other known genes. Depending on the size of proteins, they are divided into 5 families: *MGT-100*, *MGT-110*, *MGT-300*, *MGT-360*, and *MGF-505* [79, 80]. It is known that MGF proteins play an important role at different stages of viral infection and modulate transcription and translation in host cells. For example, the *MGF-360* and *MGF-505* genes have been shown to be important for the propagation of the ASFV strain BA71V in macrophages [80, 81]. However, the properties of most MGF proteins still remain unexplored.

The low pathogenicity of non-hemadsorbing isolates may be due to the loss of virulence factors due to large deletions close to the left end of the genome or smaller deletions or substitutions in genes encoding virulence factors elsewhere in the genome [62]. When strains NH/P68 and OURT88/3 were compared with strain Lisbon 60, the main differences were found in the left part of the genome (Fig.) [82]. Discrepancies were also noted in the central and right parts of the genome. The main differences were established in the genes of MGF proteins.

Comparison of the genome of the African swine fever virus strain Lisbon 60 (L60) with the genomes of strains NH/P68 (NHV) and OURT88/3. The bold black line shows homology regions of the NHV and OURT88/3 genomes with the Lisbon 60 genome. The thin black line shows deletions in the NHV and OURT88/3 genomes; the vertical black lines show insertions that are present in NHV and OURT88/3**.**

The LVR of the genomes of strains NH/P68 and OURT88/3 is 10 kb shorter than the LVR of the genome of strain Lisbon 60. Notably, in addition to the deletion, there is a 4458 bp insert located between *MGF 110*-2L and *110-13L*. The insert contains genes for proteins *MGF 110-4L*, *110-5L*, *110-9L*, *100-1R*, *ORFs 285L* and *86R*. Unfortunately, the functions of the proteins encoded by these genes are unknown. The next difference is the absence of nucleotides at positions 7244-8632 in the genome of the NH/P68 strain compared to the Lisbon 60 strain. This deletion results in the absence of the *MGF 110-11L*, *110-12L*, and *110-13L* genes. As a result of another deletion 2173 bp long, the NH/P68 strain lost the *MGF 360-6L* gene. It should be noted that this gene is also absent in the

non-pathogenic strain BA71V. The third region that is absent in the genome of the NH/P68 strain is fragment 19809-29877 of the Lisbon 60 strain genome. This deletion results in the loss or damage of the genes *MGF 360-9L, 360-10L, 360-11L, 360-12L, 360-13L, 360-14L, 505-1R, 505-2R, 505-3R.* Some of them are involved in macrophage replication, virulence, tick infection, and type I IFN immune response [82-84] (Table). The RVR regions of NH/P68 and Lisbon 60 also have differences. Basically, these are short insertions or deletions in the genes encoding MGF proteins. The protein of the virulent strain, encoded by the *MGF 360-16R* gene, is two amino acids shorter than its non-hemadsorbing phenotype homologue. The MGF 505-11L protein in the NH/P68 and OURT88/3 strains has an insert (its length is four amino acids). Non-hemadsorbing strains also have a mutation in the *MGF 100-2L* gene, which leads to a frameshift and the appearance of a stop codon [85, 86].

MGF families in the LVR and RVR genomes of the virulent hemadsorbing strain of African swine fever virus Lisbon 60 and the avirulent non-hemadsorbing strain NH/P68 (NHV) [86]

Region of the genome	*MGF* family	Strains	
		Lisbon 60	NH/P68
LVR (left)	*MGF 100*	-	1R
	MGF 110	1L, 13L+2L, 11L, 12L, 13L, 14L	1L, 2L, 4L, 5L, 9L, 13L§, 14L
	MGF 300	1L, 2R, 4L	1L, 2R, 4L
	MGF 360	1L, 2L, 3L, 4L, 6L, 8L, 9L, 10L, 11L, 12L, 13L, 14L	1L, 2L, 3L, 4L, 8L, 9L‡
	MGF 505	1R, 2R, 3R, 4R, 5R, 7R, 8R, 9R, 10R	3R‡, 4R, 5R, 7R, 8R, 9R, 10R
RVR (right)	*MGF 100*	2L	2L‡
	MGF 360	16R, 18R	16R, 17R‡, 18R
	MGF 505	11L	11L

N o t e. A dash means no members of the *MGF* family of the specified type. ‡ is a truncated version of the gene; § is different from the same gene in the OURT88/3 strain.

The CD2v glycoprotein, which is encoded by the *EP402R* gene, is responsible for the hemadsorption phenomenon during ASF virus reproduction [87, 88]. This glycoprotein is homologous to the mouse, human, and porcine T cell adhesive receptor CD2. Deletion of the *EP402R* gene from the ASF virus genome deprived it of its ability to induce the adsorption of porcine erythrocytes on the surface of infected cells, but did not affect the rate of its reproduction in vitro. Expression of CD2v on the surface of viral particles correlates with the association of virions with erythrocytes in the blood of infected pigs [88]. The calculated molecular weight of the CD2v glycoprotein polypeptide is about 45 kDa, the mature glycoprotein, taking into account carbohydrate chains, is 105-110 kDa [89, 90]. According to its hydrophilic profile, it is a typical transmembrane glycoprotein consisting of four differentiated regions: a hydrophobic leading region at the N-terminus of 20 amino acids, a hydrophilic extracellular part of 183 amino acids with 15-16 N-glycosylation sites, a transmembrane region of 25 amino acids and a proline-rich cytoplasmic C-terminal part of 174 amino acids. Comparative genomics has shown that the *EP402R* open reading frame is one of the most variable in the ASFV genome [91]. Genotyping for the genetic locus encoding CD2v coincides with the grouping of ASF virus strains according to seroimmunogroups [92, 93]. In the ASF virus strains 26544/OG10, Benin 97/1 and BA71V, the polypeptide determined by the EP402R gene consists of 428 amino acids, in the E75 strain it consists of 420 amino acids, in the non-hemadsorbing strains OURT/88 and NHV/P68 it consists of 330 [82].

It has been established that the interaction between CD2v and its ligand on erythrocytes is stabilized through the expression of a virus-specific lectin-like C-type glycoprotein encoded by the *ORF EP153R*, since the deletion of the *EP153R* gene led to a decrease in hemadsorption around ASF virus-infected cells

[85, 94].

There is an opinion that hemadsorption is not directly related to virulence, since non-hemadsorbing virulent isolates are known [95, 96]. Deletion of the *EP402R* or *EP153R* genes from the Malawi LIL20/1 isolate genome did not reduce its virulence in domestic pigs [85]. Interestingly, the haemadsorbing phenotype favors increased ASF virus replication in ticks. This was observed after recovery of haemadsorbing activity in the NHV/P68 strain, with no recovery of virulence in pigs [55]. Both glycoproteins also perform other functions: CD2v has immuno-suppressive activity (28), C-lectin-like glycoprotein suppresses apoptosis and expression of the histocompatibility antigen SLA I on the plasma membrane [92].

The question is natural of the molecular mechanisms of the origin of non-hemadsorbing strains of the ASF virus. Naturally isolated strains not capable of haemadsorption showed changes in the *EP402R* gene sequence. The loss of haemadsorbing properties in some strains of the ASF virus is associated with deletions and/or frameshifts in the *EP402R* gene [86, 97]. It can be assumed that, along with the deprivation of the ability to induce hemadsorption, the ability of non-hemadsorbing isolates to form immunotype-specific protection should be lost. In fact, this is not the case [98]. Most non-hemadsorbing isolates and laboratory strains retain the ability to induce immunotype-specific protection [99-101].

Thus, populations of African swine fever (ASF) virus and susceptible animals in Africa represent a co-evolutionary biological system. Virus isolates differ in genetic and immunobiological characteristics. To date, 24 genotypes and 9 immunoserotypes of the ASF virus have been established, as well as a variety of isolates in terms of virulence, as well as the ability to induce hemadsorption. Non-hemadsorbing isolates and strains are a natural element of the phenotypic heterogeneity of the ASF virus. The absence of haemadsorbing properties is explained by deletions and/or frame shifts in the *EP402R* gene encoding CD2v envelope glycoprotein. As a rule, isolates isolated from nature or obtained under laboratory conditions, non-hemadsorbing strains of ASF virus are characterized by low virulence up to the absence of clinical symptoms after inoculation in pigs, as well as the ability to form an immune defense against subsequent infection with homologous virulent hemadsorbing isolates. Therefore, non-hemadsorbing natural, laboratory-selected and recombinant strains of the ASF virus are used both to obtain fundamental knowledge about the mechanisms of protective immunity formation and to develop promising live vaccines against ASF.

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