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The potential of the DNA constructs was confirmed by pig immunization using ASFV DNA constructs. Immune protection at ASF is due to cytotoxic T-lymphocytes (CTL) and antibody-dependent cell-mediated cytotoxicity (ADCC) effectors against viral proteins located on infected monocyte/macrophage. There is a synergism of the immune response against ASF, achieved by administration of two recombinant proteins, p30 and p54, fused to one chain of the antibody recognizing the invariant epitope of pig swine fever virus (ASFV). The agent of African swine fever (ASF) is a large envelope virus (ASFV) belonging to family Asfarviridae and containing a double-stranded linear DNA of 170 to 190 kb in size coding for more than 150 proteins, most of which are involved in host-virus interactions (L.K. Dixon et al., 2004). Its virulent isolates cause a contagious hemorrhagic disease with 100% mortality both among domestic pigs (Sus scrofa domesticus) and wild boars (Sus scrofa). The disease control is complicated by the lack of any specific preventive methods (R.J. Rowlands et al., 2008; D.A. Chapman et al., 2011; P. Rahimi et al., 2010). The attempts to protect pigs against ASF with experimental live and inactivated subunit vaccines developed by standard methods failed (S. Blome et al., 2014). This paper discusses immunological mechanisms to provide the specific defense based on potentially protective virus-specific proteins, and immunogenic and some protective properties of ASFV gene-based DNA constructs. Immune protection at ASF is due to cytotoxic T-lymphocytes (CTL) and antibody-dependent cell-mediated cytotoxicity (ADCC) effectors against viral proteins located on infected monocyte/macrophage. There is a synergism of these effectors (A.D. Sereda, 2013). Based on i) the location, structure and functional properties of viral proteins, ii) the polypeptide specificity of blood antibodies after injecting pigs with ASFV attenuated or virulent strains, iii) the effects of pig immunization using purified proteins from infected cells or the recombinant proteins, and DNN constructs, p30, p54 and CD2v proteins are considered as potentially protective (S.D. Kollinberger et al., 2002; M.G. Barderas et al., 2001; J.G. Neilan et al., 2004). A significant disadvantage of the candidate DNA vaccine is a relatively low immune response, especially in large mammals. There were attempts of overcoming the problem using various strategies (J. Rajcanci et al., 2005, M.A. Liu et al., 2006; L.H. van Drunen et al., 2004; J.A. Leifert et al., 2004). To target the lymphocytes expressing receptors CD48 and CD58 to the protein CD2 of the antigen-presenting cells (APC), the secretory part (s) of ASFV protein HA (or CD2v) has been used (A. Brossay et al., 2003; K. Crosby et al., 2004). The addition of sHA gene to the DNA construct enhanced both humoral and cellular responses in pigs against fused recombinant proteins p30 and p54 (F. Ruiz-Gonzalvo et al., 1996). An increase in the humoral response due to targeting p30 and p54 fused to one chain of the antibody recognizing the invariant epitope of pig class II main histocompatibility complex (MHC) was demonstrated. However, the enhancement of the humoral immune response to p30 and p54 rather often resulted in earlier death of pigs infected with virulent strains. To stimulate the specific CD8+ T-cell responses, a pCMV-UbsHAPQ construct coding for antigenic determinants p30, p54 and sHA fused with cellular ubiquitin (Ub) was developed. The immunization using pCMV-UbsHAPQ did not induce an instrumentally determined antibody response although provisionally pig protection against ASFV challenge (J.M. Argilaguet et al., 2011). The potential of the DNA constructs was confirmed by pig immunization using ASFV DNA libraries (ASFVUbi) coding for viral genome short fragments combined with the cellular ubiquitin gene (A. Lacasta et al., 2014). In the 4029 clones, about 76% of the viral genome (130 kb) were covered. As many as 60% of ASFVUbi-immunized pigs survived after infection with an ASFV virulent strain. According to ELISA, none of the ASFVUbi-immunized pig had detectable specific antibodies to ASFV proteins prior to the challenge. The CD8+ T-cells comprised the only cell sub-

Abstract

The agent of African swine fever (ASF) is a large envelope virus (ASFV) belonging to family Asfarviridae and containing a double-stranded linear DNA of 170 to 190 kb in size coding for more than 150 proteins, most of which are involved in host-virus interactions (L.K. Dixon et al., 2004). Its virulent isolates cause a contagious hemorrhagic disease with 100% mortality both among domestic pigs (Sus scrofa domesticus) and wild boars (Sus scrofa). The disease control is complicated by the lack of any specific preventive methods (R.J. Rowlands et al., 2008; D.A. Chapman et al., 2011; P. Rahimi et al., 2010). The attempts to protect pigs against ASF with experimental live and inactivated subunit vaccines developed by standard methods failed (S. Blome et al., 2014). This paper discusses immunological mechanisms to provide the specific defense based on potentially protective virus-specific proteins, and immunogenic and some protective properties of ASFV gene-based DNA constructs. Immune protection at ASF is due to cytotoxic T-lymphocytes (CTL) and antibody-dependent cell-mediated cytotoxicity (ADCC) effectors against viral proteins located on infected monocyte/macrophage. There is a synergism of these effectors (A.D. Sereda, 2013). Based on i) the location, structure and functional properties of viral proteins, ii) the polypeptide specificity of blood antibodies after injecting pigs with ASFV attenuated or virulent strains, iii) the effects of pig immunization using purified proteins from infected cells or the recombinant proteins, and DNN constructs, p30, p54 and CD2v proteins are considered as potentially protective (S.D. Kollinberger et al., 2002; M.G. Barderas et al., 2001; J.G. Neilan et al., 2004). A significant disadvantage of the candidate DNA vaccine is a relatively low immune response, especially in large mammals. There were attempts of overcoming the problem using various strategies (J. Rajcanci et al., 2005, M.A. Liu et al., 2006; L.H. van Drunen et al., 2004; J.A. Leifert et al., 2004). To target the lymphocytes expressing receptors CD48 and CD58 to the protein CD2 of the antigen-presenting cells (APC), the secretory part (s) of ASFV protein HA (or CD2v) has been used (A. Brossay et al., 2003; K. Crosby et al., 2004). The addition of sHA gene to the DNA construct enhanced both humoral and cellular responses in pigs against fused recombinant proteins p30 and p54 (F. Ruiz-Gonzalvo et al., 1996). An increase in the humoral response due to targeting p30 and p54 fused to one chain of the antibody recognizing the invariant epitope of pig class II main histocompatibility complex (MHC) was demonstrated. However, the enhancement of the humoral immune response to p30 and p54 rather often resulted in earlier death of pigs infected with virulent strains. To stimulate the specific CD8+ T-cell responses, a pCMV-UbsHAPQ construct coding for antigenic determinants p30, p54 and sHA fused with cellular ubiquitin (Ub) was developed. The immunization using pCMV-UbsHAPQ did not induce an instrumentally determined antibody response although provisionally pig protection against ASFV challenge (J.M. Argilaguet et al., 2011). The potential of the DNA constructs was confirmed by pig immunization using ASFV DNA libraries (ASFVUbi) coding for viral genome short fragments combined with the cellular ubiquitin gene (A. Lacasta et al., 2014). In the 4029 clones, about 76% of the viral genome (130 kb) were covered. As many as 60% of ASFVUbi-immunized pigs survived after infection with an ASFV virulent strain. According to ELISA, none of the ASFVUbi-immunized pig had detectable specific antibodies to ASFV proteins prior to the challenge. The CD8+ T-cells comprised the only cell sub-

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MECHANISMS OF IMMUNE RESPONSE AND PROSPECTS FOR DNA VACCINES AGAINST AFRICAN SWINE FEVER
(review)


A b s t r a c t

To target the lymphocytes expressing receptors CD48 and CD58 to the protein CD2 of the antigen-presenting cells (APC), the secretory part (s) of ASFV protein HA (or CD2v) has been used (A. Brossay et al., 2003; K. Crosby et al., 2004). The addition of sHA gene to the DNA construct enhanced both humoral and cellular responses in pigs against fused recombinant proteins p30 and p54 (F. Ruiz-Gonzalvo et al., 1996). An increase in the humoral response due to targeting p30 and p54 fused to one chain of the antibody recognizing the invariant epitope of pig class II main histocompatibility complex (MHC) was demonstrated. However, the enhancement of the humoral immune response to p30 and p54 rather often resulted in earlier death of pigs infected with virulent strains. To stimulate the specific CD8+ T-cell responses, a pCMV-UbsHAPQ construct coding for antigenic determinants p30, p54 and sHA fused with cellular ubiquitin (Ub) was developed. The immunization using pCMV-UbsHAPQ did not induce an instrumentally determined antibody response although provisionally pig protection against ASFV challenge (J.M. Argilaguet et al., 2011). The potential of the DNA constructs was confirmed by pig immunization using ASFV DNA libraries (ASFVUbi) coding for viral genome short fragments combined with the cellular ubiquitin gene (A. Lacasta et al., 2014). In the 4029 clones, about 76% of the viral genome (130 kb) were covered. As many as 60% of ASFVUbi-immunized pigs survived after infection with an ASFV virulent strain. According to ELISA, none of the ASFVUbi-immunized pig had detectable specific antibodies to ASFV proteins prior to the challenge. The CD8+ T-cells comprised the only cell sub-
population among the studied ones that showed a statistically significant growth in the survived pigs starting from day 5 post immunization. The opportunities for a vaccination strategy based on the use of BacMam viruses that are baculovirus vectors encoding viral antigens under the control of cell-active promoters of vertebrates have been analyzed (J.M. Argilaguet et al., 2013). Immunization with recombinant baculovirus (BacMam-sHAPQ) encoding two ASFV full-length immunodominant proteins p30 and p54 fused to a carboxyl terminus of the extracellular domain of a viral hemagglutinin sHA resulted in no viraemia or clinical signs of the disease in 66 % of the pigs. Moreover, BacMam-sHAPQ-immunized animal had no ELISA-detectable virus-specific antibody prior to challenge. Thus, the prospect for development of DNA vaccine against ASFV seems to be encouraging.

Keywords: DNA vaccines, African swine fever, protective proteins, antibody, cytotoxic T-lymphocytes

The agent of African swine fever (ASF) is a large envelope virus (ASFV) belonging to family Asfarviridae and containing a double-stranded linear DNA of 170 to 190 kb in size coding for more than 150 proteins, most of which are involved in host-virus interactions [1]. Its virulent isolates cause a contagious hemorrhagic disease with 100 % mortality both among domestic pigs (Sus scrofa domesticus) and wild boars (Sus scrofa). First ASF outbreak was registered in 2007 in Caucasus region of Georgia [2]. From there disease had spread to Armenia, Azerbaijan, Nagorny Karabakh, Iran, Abkhazia, and Russia, and further to Ukraine, Belarus, Baltic States, and Poland [3]. The disease control is complicated by the lack of any specific preventive methods and preparations despite permanent attempts of their developing. All traditional preparations, i.e. viral strains inactivated by UV radiation, freon, ionic and nonionic detergents, β-propiolac-tone, etc., extracts from cell cultures of lung, marrow, and spleen macrophages of the infected pigs, purified virions fixed on bull erythrocytes, mycobacteria, γ-globulin, infected macrophages fixed by glutaraldehyde, etc. [4-6], were ineffective. At ASF epizooty during years 1962-1963 at Iberian Peninsula, large-scale vaccination with attenuated ASFV strains in Portugal and Spain has resulted in 3-6-fold increased number of sites at risk of ASF outbreaks, 10-50 % mortality of the vaccinated livestock, and clinical manifestation of the disease during post-vaccination and farther periods [7]. Such complications of the disease could be due to insufficient information on the attenuated strains or their antigenic non-conformity to the circulated virulent virus since there had been a report of several immunologic types of ASFV at Iberian Peninsula [8]. In USSR, laboratory attenuated strains and their various formulations (so called live vaccines) for temporary pig protection from ASFV of seroimmunotypes I-V have been obtained based on the concept accepted in the 1970s [9]. The disadvantages of live vaccines include emergence of carriers of the attenuated strain manifested to some degree, likelihood of partial restoration of vaccine strain virulence, development of subclinical infection sometimes turning into a chronic form, and insufficient protectiveness in animals with low immunity, for instance bred sows. Further infection of animals, vaccinated by attenuated strains, with virulent ASFV strains of homologous sero-immunotypes usually resulted in virus carriage in survived pigs [9-11].

These findings demonstrate that eradication of the disease requires taking strict veterinary and sanitary measures involving total slaughter of pigs in the infection foci and in the first threatened zone. Nevertheless, something is expected from DNA vaccinations. Here, we would consider immunologic special protection mechanisms, potentially protective virus-specific proteins, approaches to construction of DNA vaccinations bearing ASFV genes, as well as research findings of their immunogenic and protective properties

Immunologic mechanisms of specific protection. For development of next generation vaccines, it is important to know mechanisms of specific protection, and the role and interrelation of humoral and cell immunity at ASF. ASFV-specific antibodies are considered as important protection compo-
nent. Transfer of serum and colostrums from the survivors to intact pigs prior to their infection with corresponding virulent isolate might delay clinical manifestations, decrease viraemia, and increase the percentage of survived animals. Out of animals, which had received antiASFV immunoglobulin (Ig), 85% had survived after the infection with virulent ASFV strain E75 compared to 100% mortality in the control where pigs had received either Ig against pseudorabies or normal pig Ig, or phosphate buffer solution. In addition to the significantly delayed and poorly manifested fever, animals receiving anti-ASFV Ig remained clinically healthy after infection. There was a 10000-fold reduction of average and maximum virus titers as compared to the control group, in which clinical signs of ASF have been manifested as early as 4 days following infection [12].

It should be noted that humoral immunity response mechanisms remain debatable over a long time. Although in 1980s the opinion prevailed that ASFV does not induce neutralizing antibodies [13], based on findings published in 1990s it was hypothesized that virus neutralizing antibodies are critical in protection against ASF [14]. It has been reported on specific virus neutralizing antibodies to ASFV structural proteins p30, p54 and p72 [15]. The pigs of three groups were immunized three times with ASFV proteins p30 and p54, which were expressed in cells of insects infected by recombinant baculoviruses with inserted genes for p30 or p54 proteins. Group I was challenged with recombinant protein p30, group II was challenged with p54, and group III was challenged with combination of p30 and p54. The immunoblotting had confirmed that serum of immunized pigs contained antibodies specific to injected recombinant proteins. Within 15 days following the last immunization, the animals were intramuscularly infected with hypervirulent strain ASFV E75 in dosage of 10^2 HAU_{50}. Mortality rate in the non-immunized group reached 100% on days 5 to 6; animals immunized by either p30 or p54 died on days 5-10, in this the development of viraemia in these animals was similar. Upon combined immunization with p30 and p54, disease development widely varied. Of six infected pigs, two pigs became ill 3 days after control pigs but survived; three pigs, which became ill 10 days after the animals immunized separately by each protein, died on days 21-27 following the challenge, and one animal showed no clinical symptoms of the disease and viraemia. Only in one pigs out of three survived animals, ASFV was found by PCR on day 46 after immunization only in supramaxillary lymph nodes, whereas outcomes were negative in other samples of immune system organs. The authors have associated partial protective effect of combination of p30 and p54c proteins with their ability to induce antibodies which can prevent virion attachment or penetration into target cells [16]. The opposite hypothesis had also been practically and theoretically validated. Subject to such hypothesis, formation of immune complexes promotes penetration of virus into target cells, the monocytes (macrophages), due to activation of phagocytosis. The above-mentioned effects of serum antibodies in vivo have been associated with antibody-dependent cell-mediated cytotoxicity (ADCC) mechanism [17, 18].

It was shown that cytotoxic T-lymphocytes (CTL) are important for maintenance of virus-specific protection in the early period following ASFV infection [19, 20]. Cytolysis percentage of macrophages infected by homologous isolate was higher than that for heterologous isolates, which evidenced on immunotype specificity of CTL [21]. Critical role of virus-specific CTLs in protection against ASF was most visibly proved by the fact that in vivo exhaustion of CD8^+ T-cells by monoclonal antibodies abolishes protective immunity formed after inoculation of attenuated strain OUR/T88/3 [22].

Therefore, immune protection against ASFV is likely due to effect of
killer T-lymphocytes and ADCC against viral proteins at surface of infected monocytes (macrophages) [20, 23]. Synergy of CTLs and antibodies in ADCC had been proved by in vitro tests with the use of homologous cultures of leukocyte cells obtained before and on day 6 post injection of high dose of the attenuated ASFV strain. Antibody-mediated protection mechanisms had exceeded those of CTLs in ability to restrict ASFV reproduction [24]. It appears that virus-specific protection against ASF is ensured by not just one, but several proteins, which all induce either humoral or cell immunity effectors [25].

Potentially protective proteins. Given i) localization, structure, and functions of virus induced proteins manifested in virion envelope and cytoplasmic membrane of infected cells, ii) polypeptide specificity of pigs’ blood antibodies against virus proteins at early stages after injection with attenuated or virulent ASFV strains, iii) effect of immunization with proteins purified from the infected cells, recombinant proteins, and with DNA constructs, the p30, p54, p73 and CD2v proteins have been previously considered as potentially protective [16, 17, 25-27].

Inner membrane of ASFV virion includes proteins p12 (dimer), p22, p54 and p30, outer membrane which derived upon budding of virus particle through cell plasmatic membrane consists of p12, p24 and CD2v [28]. Proteins p12, p30 and p54 are critical at ASFV attachment and penetration into target cells. It is assumed that antibodies to p54 block virion binding to cell surface, whereas antibodies to p30 inhibit virus entering into the cell [15, 16]. Based on radioimmunoprecipitation data and immunoblotting, p30, p54 and p72 have been characterized as highly-immunogenous (inducing antibodies) [29-31]. Processes determining the results of pig ASFV infection which can be instrumentally recorded take place from day 3 to day 10. ACCD was observed within 3-6 days after high-dose inoculation of the attenuated strains FC-135, and primary CTLs were detected from day 4 to day 10. As late as on days 3-6 following injection of the attenuated, low-virulent or virulent ASFV strains, radioimmunoprecipitation had revealed 30 and 36-39 kDa polypeptide antibodies in blood which were p30 and non-glycosylated p54, respectively [17]. Less sensitive immunoassay and immunoblotting with the use of recombinant p30 as an antigen allowed detection of the antibodies since days 7-8 after infection with various virulent ASFV strains [32-34].

Proteins p30, p73, major glycoprotein GP 110-140 (or CD2v, HA) have been considered as potential antigens for CTL [35]. CTL induction might explain partial protection against ASFV after immunization with recombinant proteins p30 and p54 expressed in baculovirus [16], with hemagglutinin sHA ASFV or serotype specific HP 110-140 in liposomes [36].

Enveloped viruses, as a rule, have protective glycoprotein defining serotypical specificity. For ASFV, major glycoprotein GP 110-140 owns serotypical specificity [35]. Based on similarity of physical and chemical properties, this protein corresponds to CD2v encoded by EP402R gene, which, as we know, directly participates in hemadsorption upon ASFV infection of the susceptible cells [37]. In confirmation of this fact, it had been established that data of genotyping for locus encoding CD2v corresponded to distribution of ASFV isolates and strains by seroimmunotypes [38]. Assumedly, this protein could be main CTL inductor. Crucial role of CD2v (GP 110-140) in protective immunity upon ASF infection had been practically validated. Fourfold immunization of pigs with purified GP 110-140 in liposome composition had protected 67 % of animals from death, but not from the future repeated infection by virulent strain F-32 [36]. Immunization with recombinant baculovirus with ASFV gene for CD2v had protected against further control challenge with virulent strain. Intramuscular injection of DNA constructs containing genes for p30, p54 and extracellular domain
CD2v had prevented mortality of up to 67% pigs infected in furtherance with virulent ASFV strain [39, 40].

Thus, many researchers consider p30 and p54 proteins and CD2v as crucial elements for induction of the immune protection against ASF.

**DNA vaccines.** DNA immunization first described in 1990s [41] is used upon development of vaccines against cancer, infection and autoimmune diseases. It is conceptually important that DNA vaccines are potentially safe for animals and induce not only humoral, but also cell immunity (Fig.) [42, 43].

**Induction of cell and humoral immunity by DNA vaccinations:** SLAI and SLAII are pig antigens with histocompatibility of I and II grades; APC — antigen presenting cells; TCR and BCR — specific receptors of T- and B-cells for identification and linkage of certain antigen; B cell — B-lymphocytes; CD4 T — T-helpers; CD8T — T-suppressors (cytotoxic T-lymphocytes) (by reference 42).

Sufficient disadvantage of candidate DNA vaccines is relatively low induction of immune response, especially in big mammals. Several approaches have been tested to overcome this problem [44-48].

**Targeting.** Antigen targeting was successful in many systems [46]. First attempts to induce protective immune response against ASFV with the use of DNA constructs encoding p54 and p30 in form of fusion protein PQ were unsuccessful [49]. Similarity between ASFV hemagglutinin HA (CD2v) and molecule CD2 of leukocytes [37, 50] had allowed assumption that CD2v is capable of targeting lymphocytes which express receptors to CD2 (CD48 and CD58) toward viral antigens in antigen presenting cells [51, 52]. Addition of sHA had strengthened both humoral and cell response to PQ with optimal result after 3 intramuscular injections. Enhanced immune response to sHA injection may also be due to presence of T-helper cell epitopes in such molecule. The other strategy is based on targeting of viral antigens to the points of antigen presentation [53, 54] with the use of single-chain variable fragment (ScFv) antibodies [55], which specifically identify cell antigens on surface of antigen presenting cells [56, 57]. This strategy had appeared to be effective in induction of the immune responses against numerous different antigens both upon immunization with recombinant subunit proteins, as well as upon DNA vaccination [58, 59].

Targeting of recombinant viral antigens fused with a chain of antibody recognizing invariant epitope of MHC molecule II (APCH1) enhances immune response to subunit vaccines in mice and rabbits [59]. Potential of APCH1 had been demonstrated in vitro upon Vero cell transfection with plasmid pCMV-APCH1GFP, which encodes pig ScFv fused to green fluorescent protein (GFP). Following transfection, fused protein secreted by Vero cells was able to bind to pig macrophages in a specific manner. Effectiveness of APCH1 as a genetic adjuvant had been confirmed in vitro at immunization of pigs by plasmid pCMV-APCH1PQ, in which APCH1 gene is fused with chimeric open reading frame (ORF) for PQ [26]. DNA constructs encoding only PQ have been causing production of antibodies in high titers in mice, but not in pigs, whereas injection of
pCMV-APCH1PQ had induced in pigs both synthesis of antibodies specific to PQ, and T-helpers targeted on histocompatibility II antigen, which denoted adjuvant effect of APCH1 molecule. However, such candidate DNA vaccine, as compared to earlier described candidate subunit vaccine based on the same ASFV antigens, did not protect pigs from future ASFV infection [16, 26].

*Ubiquitinylation.* Regardless of the instrumentally registered response to immunization with pCMV-sHAPQ, pigs were not protected from control infection (clinical signs and viraemia kinetics have been indistinguishable from the same in control animals). Lack of protection had coincided with induction of specific antibodies, which had not neutralized ASFV in vitro (earlier, in vivo protection was associated with them) [16]. This had confirmed inability of the candidate DNA vaccines to induce neutralized antibodies against p30 and p54, whereas non-neutralizing antibodies, as it was found, could even aggravate development of the infection [49].

pCMV-UbsHAPQ construct encoding antigen determinants p30, p54 and sHA fused with cell ubiquitin had been designed to avoid the unwanted antibody induction and to stimulate specific CD8\(^+\)-T-cell responses. As it was expected, immunization with pCMV-UbsHAPQ did not induce humoral response in pigs, but had ensured partial protection against control infection with ASFV, thus confirming the importance of T-cell response for protection from this virus. The attained protection had not been enhanced by repeated injections of DNA vaccine that, possibly, reflects the lack of booster effect for T-cell response induced after the first immunization. In particular, upon 2-fold immunization by pCMV-UbsHAPQ only 2 of 6 pigs survived, whereas upon 4-fold immunization only one pig survived. Possibly, boosting could have negative effect on protection. According to authors, 4-fold immunization with pCMV-UbsHAPQ could have resulted in low induction of antibodies not neutralizing virus and aggravating the disease [49], which in its turn, may reduce protective effect of the induced CD8\(^+\)-T-cells.

*Expression library immunization (ELI).* ELI is considered as perspective approach to development of protection means against particularly menacing infections [60, 61]. To improve immunogenicity, one could develop modified ELI-vaccines either with targeting of encoded antibodies to induction points of the immune mechanisms or with activation of intracellular destruction and presentation of antigens [62, 63]. Protectiveness of ASFV\(^{Ubih}\), the DNA library represented by ubiquitin-fused short fragments of ASFV in plasmid pCMV-Ub for enhancing induction of specific CTLs, had been studied [64]. Obtained 4029 clones (total 130 kbp) covered nearly 76% of viral genome. Suboptimal plasmid dosage at injection in animals is 0.15 \(\mu\)g, optimal is 600 \(\mu\)g. Following challenge with strain ASFV E75, all control pigs (5 animals) died within 10 days, whereas out of 5 pigs immunized with ASFV\(^{Ubih}\) 3 animals died immediately and 2 animals died on day 10, that was later, that death of control animals had commenced. Upon re-testing, the same protection level in animals had been denoted with confirmation of the protective potential of ASFV\(^{Ubih}\). Generally, during two tests 6 of 10 immunized pigs had survived lethal challenge with ASFV. Surviving animals had lower virus titers in blood and nasal discharge as compared to ASFV\(^{Ubih}\)-immunized animals died from ASF, and also to control animals. All animals had demonstrated development of the standard symptoms, including fever, however clinical state and temperature in surviving pigs became normal on day 10 to 11 after infection, whereas final restoration had accompanied by lack of viraemia starting from day 21 after infection. Virus had not been detected in such individuals in any of the studied tissues, including pharyngeal lymph nodes, tonsils, and spleen that had been confirmed by the lack of ASF-specific macro- and micro-
scopic affections at postmortem examination. In ELISA, specific antibodies to ASFV proteins had not been found in any of the ASFV^Ubbib^-immunized individuals prior to control challenge, which had confirmed the findings for plasmids encoding ubiquitinized potentially protective proteins (p30, p54, CD2v). Among all studied cell sub-populations in surviving pigs, statistically important population growth had been found only in CD8^+^-T-cells starting from day 5, which, evidently, confirms development of specific CD8^+^-T-cell responses prior to control infection with ASFV and highlights their role in protection against ASFV. Lack of complete protection had not resulted in appearance of carrier-animals since virus titers in blood, nasal liquids, lymph nodes, tonsils and spleen of the surviving individuals had been lower than test sensitivity that had minimized the threat of ASFV transfer to susceptible recipients.

**BacMam Viruses.** Vaccination opportunities for optimization of the induced immune responses had been defined based on the use of BacMam viruses, which represent baculovirus vectors encoding virus proteins under the control of vertebrata promoters, thus ensuring high transgene expression in mammalian cells [65-67]. Although arthropod cells are hosts for baculoviruses, BacMam viruses may also promote effective transduction of vertebrata cells, in which initially baculoviruses are non-replicable. Therefore, in terms of safety BacMam viruses are suitable for use as vaccine vectors [68-73]. Effectiveness of BacMam vaccination had been demonstrated in small laboratory animals with established ability to induce humoral and cell reactions [68, 74-77].

Upon use of recombinant BacMam encoding three proteins of ASFV isolate E75 under control of the vertebrata-specific promoter pCMV [39], new recombinant baculovirus (BacMam-sHAPQ) encoded two full-size immunodominant proteins p30 and p54 fused with carboxyl terminus of extracellular domain of viral hemagglutinin (sHA; HA in positions 21-204). Prior to that, in had been demonstrated that chimeric protein (sHAPQ) had induced humoral and cell immune responses in pigs after DNA injection [40].

Expression of sHAPQ in vitro in mammals had been proven by indirect immunofluorescence in cells KOP/R transduced with the use of BacMam-sHAPQ. Immunogenicity of BacMam-sHAPQ had been determined after 3-fold injection of 10^7 BFU with 15-day interval (in control wild type of baculovirus was used). Afterwards, all animals had been infected with homologous isolate E75 at 10^2 HAU_{50}. All control animals had fallen sick, with various clinical signs of ASF and short fever peak (< 41.5 °C). On day 10, virus titers in blood achieved the maximum level and then slowly reduced until death by day 17 after infecting. In 4 of 5 pigs immunized by BacMam-sHAPQ no signs of viraemia and clinical signs of disease have been detected (prior to infecting, no one animal had virus-specific antibodies). Moreover, kinetics of specific humoral reactions after infecting with ASFV was identical in both immunized and control groups. According to authors, lack of specific antibody induction could not be due to protein expression defects or antigen specificity of fused protein, since the induced antigen (sHAPQ) had caused active humoral response upon immunization of pigs by candidate DNA vaccine [40]. Inability of BacMam-sHAPQ to induce specific antibodies is not related to the initial error of BacMam strategy since such vector is successfully used with many other antigens [73, 74, 78]. One of the causes could be due to any defect in in vivo antigen presentation to B-cells as it was indicated for other antigens [79, 80]. Induced cell responses had been assessed with the use of IFNc-ELISPOT [49], for which periphery blood mononuclear cells (PBMC) produced within 15 days following each immunization or within 17 days following challenge had been stimulated during 16 hours by isolate E75 (10^6 HAU_{50}/cm^2). As it was expected, specific responses lacked in the control prior to infecting, while specific
T-cell responses were manifested in 4 of 6 pigs immunized by BacMam-sHAPQ. It is completely coherent with data obtained with the use of attenuated ASFV strain when direct correlation had been established between the protection and induction of IFNc-secreting T-cells [11].

Thus, upon BacMam-sHAPQ vaccination, protection of pigs against sub-lethal homologous infection with ASFV is possible at absence of antibody induction. Besides, induced protection and stimulation of T-cells are directly related.

Development strategy of DNA vaccines against ASF. In veterinary, preparations for DNA vaccination of horses against Western Nile virus and salmon fish against infectious hemorrhagic necrosis have already been licensed [81]. Virus-neutralizing antibodies against herpes virus-1 in bulls, bovine diarrhea viruses, distemper, classic swine cholera, aftsosa, hepatitis B in ducks, infectious bursal disease, infectious hematopoietic necrosis, Japanese encephalitis, reproductive and respiratory syndrome in pigs, pseudo rabies, rabies, vesicular stomatitis, and hemorrhagic septicemia have been found upon immunization by candidate DNA vaccines [82, 83].

If perspectives of the classical live inactive sub-unit vaccines against ASF are comprehensible, approaches to obtainment of specific protection means based on DNA technologies require further elaboration. Development of DNA vaccine against ASF first of all requires determination of purposes and minimum requirements. Application of DNA vaccine is feasible for temporary preservation of the livestock at large pig-breeding farms in the focus of infection and first threatened area for the product processing period, as well as in African countries, where this disease is enzootic. DNA vaccine is also required due to the fact that it is impossible to fully exclude the probability of occurrence of any dangerous for humans or economically significant animal-based natural or inherent virulent ASFV strains or similar pathogens. We believe that DNA vaccine shall protect against death, contamination, viraemia upon infection by homologous virulent isolate and from its acceptance; shall not cause reactions in place of injection; at 1-2-fold injection may induce protection within 14-21 days for the term of up to six months; shall be stable upon normal conditions of storage and transportation. Effective DNA vaccine presupposes in vitro measured induction of both CTLs and antibodies which participate in ADCC, are specific to the narrow scope of protective protein epitopes and not detectable in immunofluorescent assay or neutralization reactions. Herewith, optimal relationship between the induced immune cellular and antibody-mediated protective mechanisms is critical. Use of ELI-libraries could, probably, allow for determination of protective epitopes for both CTLs and ADCC [60]. It is planned to optimize delivery methods of DNA constructs to immune competent cells, to ensure targeting of translation products and immune competent cells to antigen presenting cells, and to select immunestimulants. For that end, one should use electroporation [84], additional simulating molecules, for instance, synthetic oligodeoxynucleotides containing non-methylated CpG motifs [85] or prime-booster immunization strategy [86, 87]. Operation of the body immune system is corrected by brain and vegetative nervous system [88]. Thus, finding of the means and methods of selective effect on separate sub-populations of immune system cells for realization of the protective potential of candidate DNA vaccines is also relevant. Besides, new molecular mechanisms of virulence and protection of pathogens from the host immunity, which would be discovered during research, should also be accounted for.

Thus, mechanisms of immune response to ASFV and virus-host relationships yet require clarification. Nevertheless, research findings of development of DNA vaccine against ASFV are encouraging, including DNA constructs bearing sHA gene, pCMV-UbsHAPQ encoding antigen determinants for p30, p54 and sHA fused with cell ubiquitin, DNA-library ASFV UbLib, and use of recombinant
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