LSHTM Research Online

https://researchonline.lshtm.ac.uk

RESULTS OF Fc-PROTEIN FUSION TECHNOLOGY APPLICATION FOR VACCINE DESIGN AGAINST INFECTIOUS DISEASES OF ANIMALS AND HUMAN

(review)

E.I. KATORKINA, S.Zh. TSYBANOV, A.S. MALOGOLOVKIN

Federal Research Center for Virology and Microbiology, 1, ul. Akademika Bakuleva, pos. Vol'ginskii, Petushinskii Region, Vladimir Province, 601125 Russia, e-mail elena.fadeeva.1990@inbox.ru, cybanov@mail.ru, AMalogolovkin@vniivvim.ru (corresponding author ✉)
ORCID:
Katorkina E.I. orcid.org/0000-0003-3329-0182   Malogolovkin A.S. orcid.org/0000-0003-1352-1780
Tsybanov S.Zh. orcid.org/0000-0002-4919-3080
The authors declare no conflict of interests

A b s t r a c t

The main criteria for current vaccines design are effectiveness, efficaciousness and safety. Increasing requirements for vaccine safety and purity push forward not only classical vaccine development, but also new generation vaccine technology, including sub-unit, recombinant, antiidiotypic, DNA vaccines etc. This recombinant technology has already demonstrated its advantage, efficaciousness and safety in a large field of therapeutic and curative drug development for animal and human (**S.** Khan et al., 2016). In 2011, six novel drugs were created based on the new Fc-fusion protein technology. Most of the newly developed drugs affect receptor-ligand interactions, acting as antagonists by blocking direct receptor binding, i.e. Enbrel® (etanercept; Amgen, USA), Zaltrap® (aflibercept; Sanofi, France), Arcalyst® (rilonacept; Regeneron, USA), or as agonists for direct stimulation of receptor function which augment immune response as Amevive® (alefacept, Astellas, USA) does, or decrease immune response as Nplate® (romiplostim; Amgen, USA) does. In this review, we pay attention to the most relevant results from the last few years for virus and bacterial vaccine designed based on Fc-fusion technology. The Fc-chimeras are hybrid sequences in which Fc-fragment of IgG (Fc-IgG) and targeted therapeutic protein are fused in an entire protein molecule (V. Pechtner et al., 2017). In this fusion, the hinge region of Fc-IgG is a flexible spacer between therapeutic protein and conservative part of IgG. It helps to minimize potential negative effect of two functional domains to each other. Therapeutic drugs based on Fc-fusion proteins are divided in three types, the receptor-Fc, peptide-Fc, and monomer-Fc. The Fc-fused proteins have tremendous therapeutic potential, since Fc domain in this molecules helps to specifically augment the pharmacodynamics values. Presence of Fc-domain in hybrid molecules prolongs half elimination of protein from plasma, which extends drug therapeutic activity and slows down kidney clearance for large molecules. Here, we summarize the most significant experimental data of Fc-fusion technology application against such pathogens as human immunodeficiency virus (D. Capon et al., 1989), Ebola virus (K. Konduru et al., 2011), Dengue virus (M.Y. Kim et al., 2018), influenza virus (L. Du et al., 2011), *Mycobacterium tuberculosis* (**S.** Soleimanpour et al., 2015), classical swine fever virus (Z. Liu et al., 2017). We also discuss the critical aspects of mechanism of action, drug design and Fc-fused protein production. Targeted activation of effector systems boosts protective potential of immunogenic molecules and broadens its application. The interest of this review is focused on an application of Fc-fused proteins as potential vaccines against infectious human and animal diseases. We also briefly discuss the perspectives of Fc-fused antigens for novel effective medicine developments using African swine fever virus as an example.

Keywords: Fc fragment, human immunodeficiency virus, Ebola virus, influenza virus, tuberculosis, classical swine fever virus, African swine fever virus, vaccination

Vaccination (immunization) is one of the most effective methods to manage infectious diseases of animals and humans. The number of developed

vaccines has increased significantly in recent years [1]. In 2017, the Pharmaceutical Research and Manufacturers of America (PhRMA) has published a list of 144 infectious disease vaccines under development [2]. The main requirements for modern vaccines are their efficiency, reliability, and absence of side effects [3]. Increasing requirements for vaccine safety and purity push forward not only classical vaccine development, but also new generation vaccine technologies, i.e. sub-unit vaccines, recombinant vaccines, anti-idiotypic vaccines, DNA vaccines, etc. New vaccines must also be targeted to successfully resist highly contagious infections that could not be treated before [4].

Emergent animal infections deserve special attention because of the need to prevent epidemics and restrict ability of pathogens to pass the interspecific barrier [5]. The creation of an effective vaccine against African swine fever virus (ASFV) remains one of the most acute and important problems over the past few years in domestic and world agriculture. Numerous studies are conducted by experts in many countries (Russia, USA, UK, Germany) to develop new and safe vaccines to cope with this virus [6].

The obtained results of the use of prototype vaccines against ASF virus demonstrate the protective effect of the homologous virus but do not provide protection against ASF viruses of heterologous origin. The problems are due to the biological characteristics of ASF virus and the lack of ability of antibodies to neutralize the virus [7]. Another distinctive feature of this virus is its extreme antigenic variability and heterogeneity. The data on the protective antigens of the virus and their role in the pathogenesis of the disease are also absent. The structure of the viral envelope, which includes a large number of glycated proteins, allows the virus to "mask" antigenic determinants and evade host's immune surveillance. One of the approaches to develop the means for treatment and prevention of African swine fever was the study of the role of virus surface antigens (CD2v protein, C-lectin-like protein, P54 virus membrane protein) [6, 8].
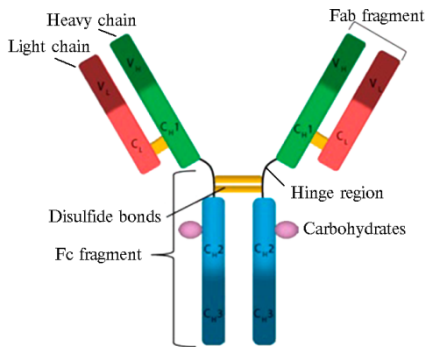
The technology of recombinant proteins has proved its advantage, effectiveness, and safety in a wide range of therapeutic and curative drugs against infectious diseases of humans and animals [9]. In 2015, the U.S. Food and Drug Administration (FDA) has approved more than 180 therapeutic fused proteins and peptides [10]. However, fused proteins have some disadvantages. Due to their small size and/or hydrolysis, these proteins are usually eliminated from the body rapidly. The short half-life of therapeutic proteins requires more frequent administration of the drug to maintain their effective concentration [11].

Two main strategies for improving the pharmacokinetics of the peptide or protein of interest exist. The first involves the formation of a repository or the introduction of an implant, which ensures the distribution of the drug from the site of introduction into the circulatory system using polymer and lipid microparticles [12]. The second is to reduce the rate of nephritic elimination of the target molecule by increasing its size [13]. It is achieved by increasing the hydrodynamic radius of the therapeutic protein by chemical conjugation with a large polymer such as polyethylene glycol (PEG) or by recombinant methods [14], as well as increasing the molecular weight of the protein to the threshold of passing through nephritic filtration (about 60-70 kDa) due to either non-covalent fusion of the therapeutic peptide with a larger carrier protein, or covalent fusion of the therapeutic peptide with the carrier protein using genetic recombination [15].

Quite often, an immunoglobulin fragment (IgG-Fc) is used as a fusion partner. A therapeutic protein fused with an IgG-Fc fragment may further provide a therapeutic effect that varies depending on the pathogenesis of the disease. Fc-fused proteins proved themselves well as therapeutic and prophylactic agents

[16]. In 2011, six drugs were created based on the Fc-fusion protein technology. Most of the Fc-fused proteins affect receptor-ligand interactions as antagonists either blocking direct receptor binding, i.e. Enbrel® (etanercept; Amgen, USA), Zaltrap® (aflibercept; Sanofi, France), Arcalyst® (rilonacept; Regeneron, USA), or as agonists for direct stimulation of receptor function which augment immune response as Amevive® (alefacept, Astellas, USA) does, or decrease immune response as Nplate® (romiplostim; Amgen, USA) does [17, 18]. The advantages of Fc-fusion drugs over other biopharmaceuticals are discussed in a number of papers [18-21]. The proteins obtained with this technology have greater therapeutic potential, as they are associated with the Fc-domain, which provides a targeted increase in the pharmacokinetics of the hybrid protein. It is proved that the presence of Fc-domain lengthens significantly the half-life of the proteins in the blood before their elimination, which prolongs therapeutic activity, and also leads to slower nephritic clearance for larger molecules [21].

This review has a particular emphasis on the most relevant recent findings in the application of Fc-fusion technology to create vaccines against viral and bacterial agents, with special attention on the prospects of this method for the development of drugs to prevent ASF.

Fig. 1. Structure of IgG antibody [10].

The functions of the Fc-fragment. Immunoglobulins G (IgG) are antibodies involved in the neutralization of bacterial and viral toxins, stimulation of phagocytosis, and complement fixation. IgG trigger the effector mechanism of the immune response by interaction with the surface of leukocytes via Fc-receptor (FcR) [22, 23]. This is the manifestation of antibody-dependent cell cytotoxicity which leads to the lysis of pathogen-infected cells by cytotoxic T-cells (cytotoxic T-cell, T-killer, CD8+) [24]. About 85% of all serum immunoglobulins (A, D, E, G, M types) are IgG (Fig. 1) [13]. As well as albumin, IgG has the longest half-life compared to other plasma proteins [25]. Due to the small molecular weight (about 150 kDa), IgG molecules diffuse freely from the vascular bed into the extracellular space where they perform a protective function. IgG can penetrate the placental barrier from the mother's blood into the fetal blood [26].

Design and structure of Fc-fused proteins. IgG is a class of antibodies that are most commonly used to treat infectious diseases. Various ligands, extracellular domains of the soluble receptor, viral antigen, etc. can act as the target peptide or protein [27]. Due to the increased size and natural IgG conversion in the body, proteins fused to the Fc-fragment are protected from degradation because of their recycling which involves the neonatal FcRn receptor.

Fc-chimeric sequences are hybrid molecules in which the Fc-fragment of IgG (Fc-IgG) and the target therapeutic protein are a single protein product derived from a gene construct. In this fusion, the FC-IgG hinge site is a flexible spacer between the therapeutic protein and the constant part of the immunoglobulin, preventing the possible negative impact of the two functional domains on each other [15, 27].

The most common types of chimeric proteins are receptor-Fc, peptide-Fc, and monomer-Fc (Fig. 2).

One of the modern approaches in the creation of vaccines leading to the in-

duction of T-lymphocytes was implemented to produce a chimeric protein consisting of an antigen and an adjuvant based on the IgG Fc-fragment [27, 28]. Such vaccines may be used as a subunit vaccine (purified protein) or as a vector carrying the gene of this chimeric protein [29].
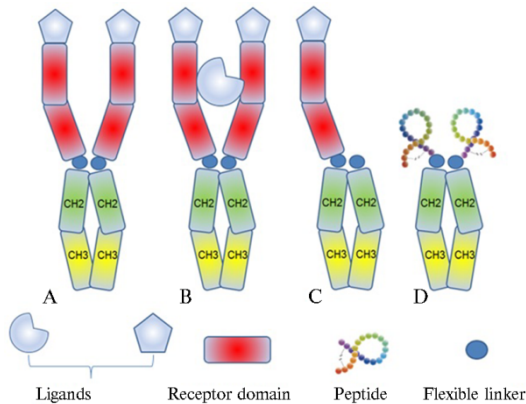
Fig. 2. Protein-protein fusion of IgG Fc-fragment with target fragments: dimeric fusion with receptor proteins (A, B; two versions of fusion with ligands), monomeric fusion with a target peptide (C), dimeric fusion with a target peptide (D).

The Fc-fusion technology has been successfully used to design vaccines against infectious diseases of humans and animals.

Fc-fused proteins as antiviral vaccines. Fc-fused proteins as vaccines against human immunodeficiency virus. The pioneering work by Capon et al. [30] on the use of Fc-fusion to develop remedies for acquired immunodeficiency syndrome (AIDS) has revealed the enormous potential of chimeric Fc-proteins for the treatment of a wide range of diseases. Capon and colleagues showed that a hybrid protein based on the extracellular part of the CD4+ receptor and Fc fragment of IgG, created for the treatment of HIV-1 (human immunodeficiency virus 1) infection, prevents the virus entering cells. Antibodies to CD4+ blocked the penetration of HIV-1 virus into T-cells, and the human cells transfected with complementary DNA (cDNA) of CD4+ became insensitive to the infection.

Soluble CD4+ (rCD4) receptor, devoid of transmembrane and cytoplasmic CD4+ sequences, can block the penetration of the HIV-1 virus into the cell, but this allows only temporary immunity to be formed [31, 32].

Fc-fused proteins as vaccines against the Ebola virus. The Ebola virus, belonging to the *Filoviridae* family, causes hemorrhagic fever in humans, which is characterized by high morbidity and mortality [33]. Filoviruses are classified as category A bioterrorism agents. The rVSV-ZEBOV vaccine is not commercially licensed but has been used under "extended access" conditions during Ebola outbreaks in North Kivu [34].

Currently, several types of recombinant vaccines based on different vectors are being developed, including adenovirus, parainfluenza virus, Venezuelan encephalitis virus, vesicular stomatitis virus, virus-like particles carrying viral glycoprotein [35]. Glycoprotein (Gp) of filovirus is the main protective antigen due to which protection against infection is provided.

In 2017, Konduru et al. [36] have reported the use of a chimeric protein of viral glycoprotein fused to the Fc-domain of immunoglobulin as a vaccine. The extracellular domain of the Zaire Ebola virus glycoprotein (ZEBOV) fused to the Fc-fragment of human immunoglobulin IgG1 (ZEBOVGP-Fc) was expressed in mammalian cells for this purpose. The results of the studies showed that the viral glycoprotein undergoes cleavage by furin [37]. Immunization of mice with the recombinant chimeric protein ZEBOVGP-Fc has activated T-cell immunity against ZEBOV virus and produced neutralizing antibodies against recombinant vesicular stomatitis virus (rVSV-GP). Mice vaccinated with the chimeric protein ZEBOVGP-Fc were protected from infection with a lethal dose of ZEBOV virus [38]. These results suggest that vaccination with only the chimeric protein ZEBOVGP-Fc may be sufficient to produce protective immunity

against the ZEBOV virus in mice.

High protection against a virulent ZEBOV virus induced by the ZEBOVGP-Fc hybrid protein indicates that a subunit vaccine based on hybrid proteins (Filovirus GP-Fc) can protect against viral infection [39]. Filo-virus GP-Fc, containing a glycoprotein bound to the Fc-domain, can be used as a standalone vaccine or in combination with other drugs such as DNA vaccines, virus-like particles or viral vector vaccines that are currently being developed. Production of the subunit vaccine based on Filovirus GP-Fc hybrid proteins is commercially quite simple, and in the case of its application the impact of side effects reduces. However, to confirm the safety of the vaccine being developed based on filovirus GP-Fc hybrid proteins, additional experiments on Guinea pigs and monkeys are necessary [40].

Fc-fused proteins as vaccines against the influenza virus. Hemagglutinins (HAs) of human influenza viruses (H1 and H3 subtypes) and avian influenza virus (H5 subtype) were obtained as recombinant proteins fused with the Fc-domain of a human immunoglobulin. Insect cells infected with baculovirus secreted recombinant HA-HuFc proteins (human influenza virus hemagglutinin fused with human Fc-domain of human immunoglobulin) as glycosylated oligomeric hemagglutinins. When mice were immunized with purified recombinant HA-HuFc protein in the absence of adjuvant, the obtained serum samples caused hemagglutination suppression, demonstrated epitope specificity and neutralized the virus. Based on the obtained results, the authors concluded that human influenza virus hemagglutinins fused with the Fc-domain of immunoglobulin can be candidate influenza vaccines [41-43].

Fc-fused proteins as vaccines against human papillomavirus. Human papillomavirus (HPV) is a huge problem in modern health care. There are 15 types of genital HPV, causing about 5% of carcinomas, primarily cervical, anogenital, and oropharyngeal transmitted sexually. All types of HPV affecting human skin tend to cause a benign form of cancer [44]. Licensed HPV vaccines based on virus-like particles carrying the main capsid antigen L1 are effective against widespread types of virus, but do not protect against other types that cause skin lesions, and are not therapeutic. Vaccines with enhanced adjuvant properties, including small capsid antigen L2, which use capsid display and fusion with early HPV antigens or Toll-like receptor antagonists, are under development.

According to Chen et al. [45], recombinant Fc-fused antigens of various viruses increase immunogenicity and induce synthesis of viral neutralizing antibodies against HPV, provide protective immunity against virulent herpes virus type II, influenza, and Ebola viruses. Chen et al. [45] showed for the first time that the fusion of HPV epitope 16 L2 (positions 17-36 bp) with a recombinant ligand for the FcRs receptor can significantly increase the immunogenicity of the L2 peptide and induce the production of cross-neutralizing antibodies and protective immunity against a number of phylogenetically distant types of human papillomavirus.

The modified Fc-fragment of human IgG1 can be used as a basis for the presentation of the L2 antigen to induce cross-neutralizing antibodies and protective immunity to different types of human papillomavirus. This type of recombinant fused protein is expressed in large quantities and can be easy purified. Therefore, the presentation of the L2 antigen together with the modified Fc-fragment provides new opportunities for the development of a vaccine against human papillomavirus [46, 47].

Fc-fused proteins as vaccines against tuberculosis. Tuberculosis caused by *Mycobacterium tuberculosis* (Mtb) ranks second in morbidity and mortality among infectious diseases worldwide [48]. As per WHO estimates, about 1.6 mil-

lion people died of tuberculosis in 2017, including 300,000 those HIV-infected [49]. An important feature of Mtb as a pathogen is its ability to survive for a long time inside the cell in a latent form that can later lead to an active tuberculous. The TB vaccine (Bacille Calmette-Guérin, BCG) is the only available licensed TB vaccine. It provides a sufficiently high protection against pulmonary tuberculosis, from 60% to 80%, but does not protect against hidden infections [50]. Consequently, the necessity for a new, safe and effective TB vaccine and an innovative vaccination strategy that could prevent all forms of TB, especially latent TB is obvious. Most new TB vaccines are currently in different stages of clinical trials or preclinical studies [51].

Simultaneous vaccination with multiple Mtb antigens can improve the protective effect against various forms of tuberculosis. Multistage fused proteins have been developed using ESAT-6 as an acute-phase antigen, with HspX protein as a latent antigen, with mouse Fcp2a fragment.

ESAT-6 antigen target is one of the most immunodominant and Mtb-specific target antigens containing multiple immunogenic T-cell epitopes capable of enhancing the cellular immune response. Mtb ESAT-6 is an important candidate antigen for the TB vaccine. In mice, Guinea pigs, and primates, TB vaccine containing ESAT-6 provides a higher protection than BCG [52].

HspX is a 16 kDa protein also known as α-crystallin Mtb that accumulates in dormant mycobacteria predominantly. It is highly immunogenic and can cause a strong cellular immune response in patients exposed to Mtb. Secreted proteins, the Mtb10.4 (Rv0288), Mtb8.4 (Rv1174c), ESAT-6 (Rv3875) and Ag85B (Rv1886c) antigens, are also highly immunogenic and can provide strong protective immunity against infection with *M. tuberculosis*, which suggests that these are promising candidate antigens [53, 54].

Profiling of immunogens to Fc-receptors (FcRs), as well as antigen-presenting cells (APCs) such as myeloid and plasmacytoid dendritic cells (DCs), monocytes, and macrophages can enhance the immune response in vitro and in vivo. This method is effective as it increases the half-life of the antigen and facilitates its uptake by APCs via FcRs and therefore increases cross-presentation efficiency for a powerful Th1 immune response. FcRI mediates selective uptake of antigens by dendritic cells, which leads to their delivery to the cytoplasm, where epitopes are recognized by the main class I histocompatibility complexes and presented to CD8+ cells. Cytotoxic T-lymphocytes (CTL) serve as an effective factor of cellular immunity for the destruction of intracellular pathogens. CTL activation via FcRs destroys an infected Mtb cell to form γ-interferon (IFN -γ), which activates infected macrophages to kill intracellular bacteria [55].

Fc-fused proteins as candidates to create a vaccine against Dengue fever. Dengue fever is an acute vector-borne viral disease that occurs with fever, intoxication, myalgia, arthralgia, rash, and lymphoid nodes enlargement. In some cases, Dengue fever develops the hemorrhagic syndrome, mainly in children under 15 years [56]. Dengue fever occurs mainly in South and South-East Asia, Africa, Oceania, and the Caribbean. The annual incidence is about 50 million people [57]. The causative agent of Dengue fever belongs to arboviruses of the *Flaviviridae* family of the *Flavivirus* genus (arboviruses of antigenic group B) [58].

The licensed Dengvaxia® vaccine (Sanofi Pasteur, France) does not protect children under the age of 9; therefore, additional vaccination strategies are necessary to stop this growing global epidemic. To obtain humanized and highly immunogenic polymeric immunoglobulin G scaffold (PIGS) fused with domain III of Dengue virus glycoprotein E (D-PIGS), the plant cell expression system was used [59]. The immunogenicity of this IgG-Fc receptor-targeted candidate vaccine has been demonstrated in transgenic mice expressing human

FcRI/CD64 [60]. In addition, recombinant molecules stimulated antigen-specific proliferation of CD4+ and CD8+ T-cells, as well as the production of neutralizing antibodies to IFN-γ. The purified D-PIGS fraction induced stronger immune activation than the monomeric form, indicating effective interaction with low-affinity Fcγ receptors on antigen-presenting cells. These results show that D-PIGS expressed in plants has the potential to be used as a single-component vaccine against Dengue fever of serotype 2 [60, 61].

A new approach to vaccination against Dengue fever is based on the application of Fc-fusion technology [62]. A design consisting of D-PIGS fused with Fc of mouse IgG2a showed high immunogenicity. To implement this approach, a version of the D-PIGS design using human immunoglobulin was created to obtain a candidate vaccine against Dengue fever. D-PIGS has shown high immunogenicity in transgenic mice expressing the human IgG receptor and, more importantly, in human tonsil cell culture. Thus, D-PIGS induced memory cell responses, IFN-γ production, and neutralizing antibodies against all four Dengue virus serotypes. This Dengue vaccine, based on human domain III and IgG1 polymer scaffold, has a potential advantage over other vaccines. It is easy to manufacture and scale, the risk of infection with animal pathogens is minimal and, most importantly, no antigenic interference, usually associated with the use of a tetravalent vaccine, is observed. The latter advantage is provided by the use of the EDIII domain III sequences of surface glycoprotein fused with human IgG1-Fc [63].

Fc-fused proteins as a vaccine against classical swine fever virus (CSFV). In recent years, a combinatorial approach based on the baculovirus vector has been widely used to create candidate vaccines against CSF virus [64]. Screening of a number of vectors based on baculovirus revealed that the baculovirus vector expressing the Fc domain of swine IgG1 has the greatest antagonism to complement (75.6%). Flow cytometry of transduced cells showed that in using this baculovirus vector the Fc-domain significantly increases the efficiency of transduction and transgenic expression of the reporter genes [65].

The E2 protein of the CSF virus was fused with the Fc-domain of swine IgG1 and translation enhancers *Syn21* and *P10UTR* were additionally linked to enhance antigen expression. The E2 protein of the CSF virus has been shown to be effectively expressed in both insect and mammalian cells. In pigs immunized with recombinant baculovirus, specific antibodies against the E2 protein responsible for the neutralization of the CSF virus, activation of the cellular immune response, and secretion of IFN-γ were synthesized with high titers. These results indicate the potential for widespread use of the Fc-domain of swine IgG1 and the surface antigen of the CSF virus [66, 67].

Thus, Fc-fusion technology has been successfully applied to the development of ways to fight many infectious diseases of viral and bacterial etiology. Structural, capsid proteins, and surface glycoproteins act as protective antigens. Both purified recombinant proteins and viral vectors (baculoviruses, adenoviruses, etc.) can provide antigen delivery [66, 68]. Regardless of the antigen delivery method, Fc-fused molecules induce strong cellular and humoral immune responses. When using recombinant Fc-fusion protein in the baculovirus system as a candidate vaccine against classical swine fever virus, intramuscular, intraperitoneal or intranasal vaccination with such constructs has been shown to induce a persistent humoral and cellular immune response. High titers of CSF-specific and neutralizing antibodies, as well as increased secretion of IFN-γ, indicate that baculovirus effectively delivers exogenous antigen to pig cells [65].

In other cases of Fc-technology application considered by the authors (Ebola, Dengue, human papillomavirus, tuberculosis causative agent), immun-

ization of animals with purified recombinant proteins was used to create antigenic constructions and effective activation of cellular and humoral immunity was also noted. In this regard, the technology of Fc-fused viral antigens as an approach to the creation of candidate vaccines looks promising in the case of ASF virus, in particular when using the viral protein CD2v, responsible for serospecificity.

So, the presented review demonstrates particular examples of the application of the proteins Fc-fusion strategy for the development of candidate vaccines against dangerous animal and human infections. Targeted activation of effectors increases the protective potential of immunogenic molecules and expands the scope of their application. Fc-fusion technology of recombinant antigens is effective to create therapeutic drugs. This approach can be promising in the development of candidate vaccines against African swine fever based on the CD2v antigen of the African swine fever virus.

R E F E R E N C E S

1. Institute of Medicine (US) Committee to Study Priorities for Vaccine Development. Progress in vaccine development. In: *Vaccines for the 21st century: a tool for decisionmaking.* K.R. Stratton, J.S. Durch, R.S. Lawrence (eds.). The National Academies Press, Washington, DC, 2000: 17-38 (doi: 10.17226/5501).
2. Shen A.K., Cooke M.T. Infectious disease vaccines. *Nature Reviews Drug Discovery*, 2018, 18: 169-170 (doi: 10.1038/d41573-018-00011-6).
3. Dellepiane N., Griffiths E., Milstien J.B. New challenges in assuring vaccine quality. *Bulletin of the World Health Organization: the International Journal of Public Health*, 2000, 78(2): 155-162.
4. Odir S., Dellagostin A. The development of veterinary vaccines: a review of traditional methods and modern biotechnology approaches. *Biotechnology Research and Innovation*, 2017, 1(1): 6-13 (doi: 10.1016/j.biori.2017.10.001).
5. Cantas L., Suer K. Review: The important bacterial zoonoses in "one health" concept. *Frontiers in Public Health*, 2014, 2: 114 (doi: 10.3389/fpubh.2014.00144).
6. Arias M., de la Torre A., Dixon L., Gallardo C., Jori F., Laddomada A., Martins C., Parkhouse R.M., Revilla Y., Rodriguez F., Sanchez-Vizcaino J.-M.. Approaches and perspectives for development of African swine fever virus vaccines. *Vaccines,* 2017, 5(4): 35 (doi: 10.3390/vaccines5040035).
7. Burmakina, G, Malogolovkin, A., Tulman, E.R., Zsak, L., Delhon, G., Diel D.G. Shobogoro N.M, Morgunov Y.P., Morgunov S.Y., Kolbasov D., Rock D. African swine fever virus serotype-specific proteins are significant protective antigens for African swine fever. *Journal of General Virology*, 2016, 97(7): 1670-1675 (doi: 10.1099/jgv.0.000490).
8. Sereda A.D., Imatdinov A.R., Dubrovskaya O.A., Kolbasov D.V. Mechanisms of immune response and prospects for DNA vaccines against African swine fever (review). *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2017, 52(6): 1069-1082 (doi: 10.15389/agrobiology.2017.6.1069eng).
9. Khan S., Ullah M.V, Siddique R., Nabi G., Manan S., Yousaf M., Hou R. Role of recombinant DNA technology to improve life. *International Journal of Genomics*, 2016, 2016: Article ID 2405954 (doi: 10.1155/2016/2405954).
10. Pechtner V., Karanikas C.A., García-Pérez L.E., Glaesner W. A new approach to drug therapy: Fc-fusion technology. *Primary Health Care*, 2017, 7: 255 (doi: 10.4172/2167-1079.1000255).
11. Strohl W.R. Fusion proteins for half-life extension of biologics as a strategy to make Biobetters. *BioDrugs,* 2015, 29(4): 215-239 (doi: 10.1007/s40259-015-0133-6).
12. Chen X., Zaro J., Shen W.C. Fusion protein linkers: effects on production, bioactivity, and pharmacokinetics. *Advanced Drug Delivery Reviews*, 2013, 65(10): 1357-1369 (doi: 10.1016/j.addr.2012.09.039).
13. Unverdorben F., Richter F., Hutt M., Seifert O., Malinge P., Fischer N., Kontermann R.E. Pharmacokinetic properties of IgG and various Fc fusion proteins in mice. *MAbs*, 2016, 8(1): 120-128 (doi: 10.1080/19420862.2015.1113360).
14. Levin D., Golding B., Strome S.E. Fc fusion as a platform technology: potential for modulating immunogenicity. *Trends in Biotechnology*, 2015, 33(1): 27-34 (doi: 10.1016/j.tibtech.2014.11.001).
15. Zvonova E.A., Tyurin A.A., Solov'ev A.A., Goldenkova-Pavlova I.V. *Uspekhi sovremennoi biologii*, 2017, 4(137): 398-419 (doi: 10.7868/S004213241704007X) (in Russ.).
16. Czajkowsky D.M., Hu J., Shao Z., Pleass R.J. Fc-fusion proteins: new developments and future perspectives. *EMBO Molecular Medicine*, 2012, 4(10): 1015-1028 (doi: 10.1002/emmm.201201379).
17. Nelson A., Reichert J. Development trends for therapeutic antibody fragments. *Nature Biotechnol*ogy, 2009, 27(4): 331-337 (doi: 10.1038/nbt0409-331).

18. Strohl W.R. Optimization of Fc-mediated effector functions of monoclonal antibodies. *Current Opinion in Biotechnology*, 2009, 20(6): 685-691 (doi: 10.1016/j.copbio.2009.10.011).

19. Strohl W.R., Knight D.M. Discovery and development of biopharmaceuticals: current issues. *Current Opinion in Biotechnology*, 2009, 20(6): 668-672 (doi: 10.1016/j.copbio.2009.10.012).

20. Reichert J. Antibody-based therapeutics to watch in 2011. *MAbs*, 2011, 3(1): 76-99 (doi: 10.4161/mabs.3.1.13895).

21. Beck A., Reichert J. Therapeutic Fc-fusion proteins and peptides as successful alternatives to antibodies. *MAbs*, 2011, 3(5): 415-416 (doi: 10.4161/mabs.3.5.17334).

22. Dumont J., Low S., Peters R., Bitonti A. Monomeric Fc fusions: impact on pharmacokinetic and biological activity of protein therapeutics. *BioDrugs*, 2006, 20(3): 151-160 (doi: 10.2165/00063030-200620030-00002).

23. Ye L, Zeng R., Bai Y., Roopenian D.C., Zhu X. Efficient mucosal vaccination mediated by the neonatal Fc receptor. *Nature Biotechnol*ogy, 2011, 29(2): 158-163 (doi: 10.1038/nbt.1742).

24. Congy-Jolivet N., Probst A., Watier H., Thibault G. Recombinant therapeutic monoclonal antibodies: mechanisms of action in relation to structural and functional duality. *Critical Reviews in Oncology/Hematology*, 2007, 64(3): 226-233 10.1016/j.critrevonc.2007.06.013).

25. Curtis J., Bourne F.J. Half-lives of immunoglobulins IgG, IgA and IgM in the serum of new-born pigs. *Immunology*, 1973, 24(1): 147-155.

26. Rath T., Baker K., Dumont J.A., Peters R.T., Jiang H., Qiao S.W., Lencer W.I., Pierce G.F., Blumberg R.S. Fc-fusion proteins and FcRn: structural insights for longer-lasting and more effective therapeutics. *Current Opinion in Biotechnology*, 2015, 35(2): 235-254 (doi: 10.3109/07388551.2013.834293).

27. Ghose S., Hubbard B., Cramer S.M. Binding capacity differences for antibodies and Fc-fusion proteins on protein A chromatographic materials. *Biotechnology and Bioengineering*, 2007, 96(4): 768-779 (doi: 10.1002/bit.21044).

28. Li F., Ravetch J.V. Inhibitory Fcγ receptor engagement drives adjuvant and anti-tumor activities of agonistic CD40 antibodies. *Science*, 2011, 333(6045): 1030-1034 (doi: 10.1126/science.1206954).

29. Stapleton N.M., Andersen J.T., Stemerding A.M., Bjarnarson S.P., Verheul R.C., Gerritsen J., Zhao Y., Kleijer M., Sandlie I., de Haas M., Jonsdottir I., van der Schoot C.E., Vidarsson G. Competition for FcRn-mediated transport gives rise to short half-life of human IgG3 and offers therapeutic potential. *Nature Communications*, 2011, 2: 599 (doi: 10.1038/ncomms1608).

30. Capon D.J., Chamow S.M., Mordenti J., Marsters S.A., Gregory T., Mitsuya H., Byrn R.A., Lucas C., Wurm F.M., Groopman J.E. Designing CD4 immunoadhesins for AIDS therapy. *Nature*, 1989, 337(6207): 525-531 (doi: 10.1038/337525a0).

31. Ratcliff A., Arts E. HIV-1 entry, inhibitors, and resistance. *Viruses*, 2010, 2(5): 1069-1105 (doi: 10.3390/v2051069).

32. Dennison S., Anasti K., Jaeger F., Stewart S., Pollara J., Liu P., Kunz E., Zhang R., Vandergrift N., Permar S., Ferrari G., Tomaras G., Bonsignori M., Michael N., Kim J., Kaewkungwal J., Nitayaphan S., Pitisuttithum P., Rerks-Ngarm S., Liao H.X., Haynes B.F., Alam S.M. Vaccine-induced HIV-1 envelope gp120 constant region 1-specific antibodies expose a CD4-inducible epitope and block the interaction of HIV-1 gp140 with galactosylceramide. *Journal of Virology*, 2014, 88(16): 9406-9417 (doi: 10.1128/JVI.01031-14).

33. Feldmann H., Geisbert T.W. Ebola haemorrhagic fever. *Lancet*, 2011, 377(9768): 849-862 (doi: 10.1016/S0140-6736(10)60667-8).

34. Henao-Restrepo A.M., Camacho A., Longini I. Efficacy and effectiveness of an rVSV-vectored vaccine in preventing Ebola virus disease: final results from the Guinea ring vaccination, open-label, cluster-randomised trial. *Lancet*, 2017, 389(10068): 505-518 (doi: 10.1016/S0140-6736(16)32621-6).

35. Towner J.S., Sealy T.K., Khristova M.L., Albarico C.G., Conlan S., Reeder S.A., Quan P.L., Lipkin W.I., Downing R., Tappero J.W., Okware S., Lutwama J., Bakamutumaho B., Kayiwa J., Comer J.A., Rollin P.E., Ksiazek T.G., Nichol S.T. Newly discovered Ebola virus associated with hemorrhagic fever outbreak in Uganda. *PLoS Pathogens*, 2008, 4(11): e1000212 (doi: 10.1371/journal.ppat.1000212).

36. Konduru K., Bradfute S.B., Jacques J., Manangeeswaran M., Nakamura S., Morshed S., Wood S.C., Bavari S., Kaplan G.G. Ebola virus glycoprotein Fc fusion protein confers protection against lethal challenge in vaccinated mice. *Vaccine*, 2011, 29(16): 2968-2977 (doi: 10.1016/j.vaccine.2011.01.113).

37. Jeffers S., Sanders D., Sanchez A. Covalent modifications of the Ebola virus glycoprotein. *Journal of Virology*, 2002, 76(24): 12463-12472 (doi: 10.1128/JVI.76.24.12463-12472.2002).

38. Takada A., Robison C., Goto H., Sanchez A., Murti K.G., Whitt M.A., Kawaoka Y. A system for functional analysis of Ebola virus glycoprotein. *PNAS USA*, 1997, 94(26): 14764-14769 (doi: 10.1073/pnas.94.26.14764).

39. Jones S.M., Feldmann H., Ströher U., Geisbert J.B., Fernando L., Grolla A., Klenk H.D., Sullivan N.J., Volchkov V.E., Fritz E.A., Daddario K.M., Hensley L.E., Jahrling P.B., Geis-

bert T.W. Live attenuated recombinant vaccine protects nonhuman primates against Ebola and Marburg viruses. *Nature Medicine*, 2005, 11(7): 786-790 (doi: 10.1038/nm1258).

40. Sullivan N.J., Geisbert T.W., Geisbert J.B., Xu L., Yang Z.Y., Roederer M., Koup R.A., Jahrling P.B., Nabel G.J. Accelerated vaccination for Ebola virus haemorrhagic fever in non-human primates. *Nature*, 2003, 424(6949): 681-684 (doi: 10.1038/nature01876).

41. Du L., Leung V.H., Zhang X., Zhou J., Chen M., He W., Zhang H.Y., Chan C.C., Poon V.K., Zhao G., Sun S., Cai L., Zhou Y., Zheng B., Jiang S. A recombinant vaccine of H5N1 HA1 fused with foldon and human IgG Fc induced complete cross-clade protection against divergent H5N1 viruses. *PLoS ONE*, 2011, 6(1): e16555 (doi: 10.1371/journal.pone.0016555).

42. Price G.E., Soboleski M.R., Lo C.Y., Misplon J.A., Pappas C., Houser K.V., Tumpey T.M., Epstein S.L. Vaccination focusing immunity on conserved antigens protects mice and ferrets against virulent H1N1 and H5N1 influenza A viruses. *Vaccine*, 2009, 27(47): 6512-6521 (doi: 10.1016/j.vaccine.2009.08.053).

43. Loureiro S., Ren J., Phapugrangkul P., Colaco C., Bailey C., Shelton H., Molesti E., Temperton N., Barclay W., Jones I. Adjuvant-free immunization with hemagglutinin-Fc fusion proteins as an approach to influenza vaccines. *Journal of Virology*, 2011, 85(6): 3010-3014 (doi: 10.1128/JVI.01241-10).

44. Bernard H.U., Burk R.D., Chen Z., van Doorslaer K., zur Hausen H., de Villiers E.M. Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. *Virology*, 2010, 401(1): 70-79 (doi: 10.1016/j.virol.2010.02.002).

45. Chen X., Liu H., Zhang T., Liu Y., Xie X., Wang Z., Xu X. A vaccine of L2 epitope repeats fused with a modified IgG1 Fc induced cross-neutralizing antibodies and protective immunity against divergent human papillomavirus types. *PLoS ONE*, 2014, 9(5): e95448 (doi: 10.1371/journal.pone.0095448).

46. Kemp T.J, Hildesheim A., Safaeian M., Dauner J.G., Pan Y., Porras C., Schiller J.T., Lowy D.R., Herrero R., Pinto L.A. HPV16/18 L1 VLP vaccine induces cross-neutralizing antibodies that may mediate cross-protection. *Vaccine*, 2011, 29(11): 2011-2024 (doi: 10.1016/j.vaccine.2011.01.001).

47. Alphs H.H., Gambhira R., Karanam B., Roberts J.N., Jagu S., Schiller J.T., Zeng W., Jackson D.C., Roden R.B. Protection against heterologous human papillomavirus challenge by a synthetic lipopeptide vaccine containing a broadly cross-neutralizing epitope of L2. *PNAS USA*, 2008, 105(15): 5850-5855 (doi: 10.1073/pnas.0800868105).

48. Khiavi F., Arashkia A., Golkar M., Nasimi M., Roohvand F., Azadmanesh K. A dual-type L2 11-88 peptide from HPV types 16/18 formulated in Montanide ISA 720 induced strong and balanced Th1/Th2 immune responses, associated with high titers of broad spectrum cross-reactive antibodies in vaccinated mice. *Journal of Immunology Research,* 2018: 9464186 (doi: 10.1155/2018/9464186).

49. *Global Tuberculosis Report 2018.* World Health Organization, Geneva, 2018. Available http://www.unaids.org/ru/resources/presscentre/featurestories/2018/september/tb-and-hiv. No date.

50. Soleimanpour S., Farsiani H., Mosavat A., Ghazvin K., Eydgahi M., Sankian M., Sadeghian H., Meshkat Z., Rezaee1 S.A. APC targeting enhances immunogenicity of a novel multistage Fc-fusion tuberculosis vaccine in mice. *Applied Microbiology and Biotechnology*, 2015, 99: 10467-10480 (doi: 10.1007/s00253-015-6952-z).

51. O'Garra A., Redford P.S., McNab F.W., Bloom C.I., Wilkinson R.J., Berry M.P. The immune response in tuberculosis. *Annual Review of Immunology*, 2013, 31: 475-527 (doi: 10.1146/annurev-immunol-032712-095939).

52. Ohara N. Current status of tuberculosis and recombinant bacillus Calmette-Guérin vaccines. *Journal of Oral Biosciences*, 2012, 54(2): 92-95 (doi: 10.1016/j.job.2012.04.002).

53. Xin Q., Niu H., Li Z., Zhang G., Hu L., Wang B., Li J., Yu H., Liu W., Wang Y., Da Z., Li R., Xian Q., Wang Y., Zhang Y., Jing T., Ma X. Zhu B. Subunit vaccine consisting of multi-stage antigens has high protective efficacy against *Mycobacterium tuberculosis* infection in mice. *PLoS ONE*, 2013, 8(8): e72745 (doi: 10.1371/journal.pone.0072745).

54. Jee B., Singh Y., Yadav R., Lang F. Small heat shock protein 16.3 of *Mycobacterium tuberculosis*: after two decades of functional characterization. *Cellular Physiology and Biochemistry*, 2018, 49(1): 368-380 (doi: 10.1159/000492887).

55. Taylor J.L., Wieczorek A., Keyser A.R., Grover A., Flinkstrom R., Karls R.K., Bielefeldt-Ohmann H., Dobos K.M., Izzo A.A. HspX-mediated protection against tuberculosis depends on its chaperoning of a mycobacterial molecule. *Immunology and Cell Biology,* 2012, 90(10): 945-954 (doi: 10.1038/icb.2012.34).

56. Bhatt S., Gething P.W., Brady O.J., Messina J.P., Farlow A.W., Moyes C.L., Drake J.M., Brownstein J.S., Hoen A.G., Sankoh O., Myers M.F., George D.B., Jaenisch T., Wint G.R., Simmons C.P., Scott T.W., Farrar J.J., Hay S.I. The global distribution and burden of Dengue. *Nature*, 2013, 496(7446): 504-507 (doi: 10.1038/nature12060).

57. *Pigmented ethnic skin and imported dermatoses: a text-atlas.* C. Orfanos, C. Zouboulis, C. Assaf (eds.). Springer International Publishing, 2018 (doi: 10.1007/978-3-319-69422-1).

58. Kim M.Y., Copland A., Nayak K., Chandele A., Ahmed M.S., Zhang Q., Diogo G.R., Paul M.J., Hofmann S., Yang M.S., Jang Y.S., Ma J.K., Reljic R. Plant expressed Fc-fusion protein tetravalent Dengue vaccine with inherent adjuvant properties. *Plant Biotechnology Journal,* 2018, 16(7): 1283-1294 (doi: 10.1111/pbi.12869).

59. Brewoo J.N., Kinney R.M., Powell T.D., Arguello J.J., Silengo S.J., Partidos C.D., Huang C.Y., Stinchcomb D.T., Osorio J.E. Immunogenicity and efficacy of chimeric Dengue vaccine (DENVax) formulations in interferon-deficient AG129 mice. *Vaccine*, 2012, 30(8): 1513-1520 (doi: 10.1016/j.vaccine.2011.11.072).

60. Kim M.Y., Van Dolleweerd C., Copland A., Paul M.J., Hofmann S., Webster G.R., Julik E., Ceballos-Olvera I., Reyes-Del Valle J., Yang M.S., Jang Y.S., Reljic R., Ma J.K. Molecular engineering and plant expression of an immunoglobulin heavy chain scaffold for delivery of a Dengue vaccine candidate. *Plant Biotechnology Journal,* 2017, 15(12): 1590-1601 (doi: 10.1111/pbi.12741).

61. De Alwis R., Smith S.A., Olivarez N.P., Messer W.B., Huynh J.P., Wahala W.M., White L.J., Diamond M.S., Baric R.S., Crowe J.E., de Silva A.M. Identification of human neutralizing antibodies that bind to complex epitopes on Dengue virions. *PNAS USA*, 2012, 109(19): 7439-7444 (doi: 10.1073/pnas.1200566109).

62. Kim M.Y., Kim B.Y., Oh S.M., Reljic R., Jang Y.S., Yang M.S. Oral immunization of mice with transgenic rice calli expressing cholera toxin B subunit fused to consensus Dengue cEDIII antigen induces antibodies to all four Dengue serotypes. *Plant Biotechnology Journal,* 2016, 92(3): 347-356 (doi: 10.1007/s11103-016-0517-0).

63. Tripathi N.K., Shrivastava A. Recent developments in recombinant protein−based Dengue vaccines. *Frontiers in Immunology*, 2018, 9: 1919 (doi: 10.3389/fimmu.2018.01919).

64. Ji W., Guo Z., Ding N.Z., He C.Q. Studying classical swine fever virus: making the best of a bad virus. *Virus Research*, 2015, 197: 35-47 (doi: 10.1016/j.virusres.2014.12.006).

65. Liu Z., Liu Y., Zhang Y., Yang Y., Ren J., Zhang X., Du E. Surface displaying of swine IgG1 Fc enhances baculovirus-vectored vaccine efficacy by facilitating viral complement escape and mammalian cell transduction. *Veterinary Research*, 2017, 48(1): 29 (doi: 10.1186/s13567-017-0434-5).

66. Martyn J.C., Cardin A.J., Wines B.D., Cendron A., Li S., Mackenzie J., Powell M., Gowans E.J. Surface display of IgG Fc on baculovirus vectors enhances binding to antigen-presenting cells and cell lines expressing Fc receptors. *Archives of Virology,* 2009, 154(7): 1129-1138 (doi: 10.1007/s00705-009-0423-8).

67. Renson P., Le Dimna M., Keranflech A., Cariolet R., Koenen F., Le Potier M-F. CP7_E2alf oral vaccination confers partial protection against early classical swine fever virus challenge and interferes with pathogeny-related cytokine responses. *Veterinary Research*, 2013, 44(1): 9 (doi: 10.1186/1297-9716-44-9).

68. Nascimento I.P., Leite L.C.C. Recombinant vaccines and the development of new vaccine strategies. *Brazilian Journal of Medical and Biological Research*, 2012, 45(12): 1102-1111 (doi: 10.1590/S0100-879X2012007500142).