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Brief report

Modern adjuvants do not enhance the efficacy of an inactivated African swine fever virus vaccine preparation

Sandra Blome*, Claudia Gabriel, Martin Beer

Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Südufer 10, 17493 Greifswald, Insel Riems, Germany

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ABSTRACT

African swine fever (ASF) is among the most devastating viral diseases of pigs. In recent years, the disease has spread alarmingly. Despite intensive research activities, promising vaccine candidates are still lacking. For this reason, a study was undertaken to re-assess inactivated ASFV preparations with state-of-the-art adjuvants. Inactivated preparations of ASF virus (ASFV) "Armenia08" were adjuvanted with either Polygen™ or Emulsigen®-D, respectively, and used to immunize six weaner pigs two times with a three-week interval. Six weeks after the first immunization, animals were challenged with the homologues highly virulent ASFV. Although ASFV-specific antibodies were detectable in all but one vaccinated animal prior to challenge, no protective effect of immunization was observed. All animals developed acute-lethal ASF and had to be euthanized within eleven days post challenge. A slightly accelerated clinical course in vaccinees could even indicate an antibody dependent enhancement, which could also influence efficacy of other vaccine approaches.

1. Introduction

African swine fever (ASF) is among the most devastating and complex viral diseases of pigs [1]. The disease, which is endemic in many countries of Sub-Saharan Africa, has recently spread to Trans-Caucasian countries and the Russian Federation [2] where it keeps occurring in domestic pigs and wild boar. Recently, it has been detected in Belarus, Ukraine, Lithuania, and Poland (WAHID, February 22nd 2014). This alarming situation and the clear tendency to spread to free areas, led to the necessity to revisit existing contingency plans. Control measures have to rely on sanitary measures, as no effective vaccines exist [1,3]. Despite intensive research activities, vaccine candidates that have potential for licensing in the near future are lacking. Traditional inactivated vaccines against ASFV were shown to lack efficacy more than 25 years ago [4,5], although a protective effect could be observed in some cases [6,7]. Since then, considerable improvements have been made regarding adjuvants, especially with action towards cellular immunity, which is vital for the protection against ASFV [8]. To exclude the possibility that there could be a traditional approach combined with modern technology, a study was undertaken to re-assess inactivated ASFV preparations with state-of-the-art adjuvants.

2. Material and methods

2.1. Experimental settings

The study comprised a total of 15 cross-bred domestic weaner pigs of approximately eight weeks of age. All animals were kept under appropriate high-containment conditions and received a commercial feed for pigs of their age class once a day combined with hay cobs. Access to water was provided *ad libitum*. All applicable animal welfare regulations and standards were taken into account (permission with reference number 7221.3-1.1-018/12). The experimental setup including the sampling scheme is depicted in Table 1. Intramuscular injections were carried out deep into the muscles behind the ear using a 2 ml syringe and a 20G needle.

For the duration of the trial, rectal body temperature was measured on a daily basis. Fever was defined as a body temperature ≥40.0 °C for at least two consecutive days. Clinical signs were recorded daily. Euthanasia was performed based on a humane end point system to prevent unacceptable suffering. At the end of the trial, all animals were subjected to post-mortem examinations.

2.2. Vaccine preparations and challenge viruses

The virus used in this trial, "Armenia08", was isolated from a diagnostic specimen sent by the Central Veterinary Laboratory, Yerevan, Armenia, in February 2008. For immunization purposes, the virus was grown on macrophages derived from

* Corresponding author. Tel.: +49 38351 7 1144; fax: +49 38351 7 1226.
E-mail addresses: sandra.blome@fli.bund.de (S. Blome), claudia@softwarebank.de (C. Gabriel), martin.beer@fli.bund.de (M. Beer).

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Table 1
Experimental setup. i.m. = intramuscularly, BEI = binary ethyleneimine, $TCID_{50}$ = tissue culture infectious doses 50%, dpv = days post vaccination, dpc = days post challenge.

Group	Animals	Immunization/adjuvant	Challenge	Sampling
T01	6 Domestic weaner pigs (app. 8 weeks of age)	Double immunization with a 3 week interval (5 ml i.m. injection) BEI-inactivated **ASFV "Armenia08"/Polygen**™ (20% v/v)	**ASFV "Armenia08"** 10^9 $TCID_{50}$/ml, i.m. 2 ml spleen suspension, 41 dpv	Serum at 0 dpv, 7 dpv, 14 dpv, 21 dpv, 28 dpv, 35 dpv, 41 dpv, 5 dpc, 8 dpc, 11 dpc EDTA blood samples at 41 dpv, 5 dpc, 8 dpc, 11 dpc Blood, spleen, tonsil, and salivary gland samples at necropsy
T02	6 Domestic weaner pigs (app. 8 weeks of age)	Double immunization with a 3 week interval (5 ml i.m. injection) BEI-inactivated **ASFV "Armenia08"/Emulsigen D** (20% v/v)		
T03	3 Domestic weaner pigs (app. 8 weeks of age)	Challenge controls		

porcine periphery blood monocytes (PBMC) to high titers (>$10^{6.5}$ tissue culture infectious doses 50% per ml). Thereafter, virus suspensions obtained after a freeze–thaw cycle were inactivated using binary ethyleneimine (BEI) according to standard procedures [9]. Inactivated preparations were either mixed with Polygen™ (MVP Technologies, Omaha, USA), a co-polymer based adjuvant, or Emulsigen®-D (MVP Technologies, Omaha, USA), an oil-in-water emulsion with depot effect that incorporates dimethyldioctadecylammonium bromide (DDA) as T-cell stimulant (20% v/v).

For challenge infection, a spleen suspension was produced using spleen material from a previous animal trial.

2.3. Laboratory investigations

Virus detection was carried out on all blood samples collected upon challenge infection, and on spleen samples taken at necropsy using virus isolation techniques on PBMC derived macrophages. Viral genome was detected in blood samples collected upon challenge infection, and in samples of blood, spleen, tonsil and salivary gland collected at necropsy by real-time polymerase chain reaction (qPCR).

Blood for the preparation of PBMC-derived macrophages was collected from domestic donor pigs. Cells were prepared as previously described [10,11] and used for virus titrations and hemadsorption tests. Both methods were carried out according to standard procedures [12]. For quantitative real-time PCR (qPCR), viral DNA was extracted using the QIAamp® DNA Mini Kit (QIAGEN) according to the manufacturer's instructions. Subsequently, qPCR was performed according to the protocol published by King et al. [13] using a Bio-Rad CFX Cycler (Bio-Rad Laboratories). A dilution series of a synthetic standard with known copy numbers was used to quantify genome copies in the respective samples. To confirm integrity of the p73 antigen after inactivation, immunogen preparations were tested in the commercial antigen ELISA Ingezim PPA DAS K2 (INGENASA) according to the manufacturer's instructions. Moreover, genome copy numbers were compared prior and after inactivation.

Sera were tested for the presence of ASFV p73- and p30-specific antibodies using the INGEZIM PPA COMPAC ELISA (Ingenasa) and the SVANOVIR ASFV-Ab (SVANOVA) according to the manufacturer's instructions. To assess neutralizing capacities of sera obtained prior to challenge infection, macrophage infection inhibition assays were carried out along with neutralization tests on permanent wild boar lung cells (WSL). Hemadsorption inhibition and indirect immunofluorescence staining was used as read-out (methodological details are available from the authors upon request).

3. Results and discussion

The presented study was undertaken to re-assess inactivated ASFV vaccines in combination with state-of-the-art adjuvants, namely "Polygen" and "Emulsigen D". These adjuvants are known to stimulate not only humoral but also cellular immune responses including interferon gamma reactions [14–16] that are crucial for protection against ASFV [8]. The virus strain chosen for this study presents the genotype II isolates currently circulating in the Russian Federation and neighbouring countries.

Upon immunization, all animals developed ASFV-specific antibodies that resulted in positive or doubtful reactions in both antibody ELISAs (results of the p73 antibody ELISA are depicted in Fig. 1). Strongest reactions were seen in animals vaccinated with Emulsigen D adjuvanted virus preparations (≥80% inhibition in the p73 antibody ELISA, and Percent Positivity (*PP*) values over 95 in the p30 antibody ELISA). Emulsigen D contains DDA as additional immunostimulant that was previously shown to induce strong responses in pigs [17] what could explain the superiority in humoral responses. Following challenge infection with the homologues ASFV, no protective effect was observed irrespective of the vaccine preparation. All animals developed an acute lethal course of ASF with high fever (see Fig. 2), depression, reduced feed intake, slight central nervous signs, and conjunctivitis. Some animals showed in addition gastro-intestinal and respiratory signs. All animals succumbed to infection within 12 days and showed pathomorphological lesions indicative for ASF including enlarged and haemorrhagic gastro-hepatic lymphnodes and lung oedema. Virus and genome detection was comparable among animals with strong positive reactions from 5 dpc (data not shown). Following challenge infection, antibodies dropped in several immunized animals indicating consumption. These reactions were most pronounced in group T01 at 5 dpc. In this group only one animal remained doubtful at 5 dpc (see Fig. 1). All but one of the vaccinated animals was positive by the day of euthanasia. No antibodies could be detected in the controls (see Fig. 1).

The overall outcome is even in contrast to previous studies that observed at least partial protection, sometimes in the majority of animals [7]. Among the possible explanation for this discrepancy could be the presentation of antigens as well as the used virus strain. Bommeli et al. [7] used a non-ionic detergent for inactivation while BEI was used in the presented study. If BEI would lead to higher fragmentation of antigens, the lack of protection could be explained. However, humoral responses indicate that at least B-cell epitopes were rather unaffected, and also the antigen ELISA indicated that major antigens were still intact.

Taking body temperature into consideration, a slightly accelerated clinical course was observed in most vaccinees (see Fig. 2). These animals were the ones with highest antibody responses and thus the ones that were expected to have reacted in a beneficial way. One explanation could be an antibody dependent enhancement that could however not be further investigated in the framework of this study due to the lack of sampling in the critical period (days 2 to 4). This phenomenon is known for several viruses and was also discussed for ASFV [18]. In consequence, humoral responses against ASFV seem to play a two-sided role. It is known