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Evaluation of protection induced by immunisation of domestic pigs with deletion mutant African swine fever virus BeninΔMGF by different doses and routes

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ABSTRACT

A live attenuated African swine fever virus (ASFV) vaccine candidate, produced by deletion of several genes belonging to multi-gene families MGF360 and 505 from virulent Benin 97/1 strain (BeninΔMGF), induces protection in pigs against parental virulent strain. In order to better define the safety and efficacy of this attenuated vaccine candidate and to understand protective mechanisms, we extended previous studies by intramuscular immunisation of pigs with the deletion mutant BeninΔMFG at different doses (10^2, 10^3, 10^4 $TCID_{50}$), together with intranasal immunisation at the 10^3 dose. Results demonstrated a strong correlation between both doses and routes of immunisation of BeninΔMFG and the percentage of protection achieved, the onset of clinical signs, the viremia levels reached and the onset of death in non-protected pigs. The results show that the intramuscular route using high doses (10^4 $TCID_{50}$) is the best option for immunisation. Only transient increase in temperature associated with a peak of virus genome levels was observed in most pigs after immunisation. Then, virus genome levels progressively decreased throughout the experiment until reaching low or undetectable levels in those protected pigs that survived after challenge. The IgM antibody responses following immunisation were detected between day 7–10 post-immunisation and remained at elevated levels for 10–18 days in most pigs before dropping. IgG was detected from day 15 to 21 post-immunisation and maintained at increased levels for the remainder of the experiment in most pigs. Induction of IFNγ and IL-10 was detected by ELISA in sera from some pigs immunised with 10^3 $TCID_{50}$ by intramuscular or intranasal route at early times post-immunisation. IL-10 was also detected in serum from some non-protected pigs included in these groups after challenge.

1. Introduction

African swine fever (ASF) is one of the most significant infectious diseases affecting the swine industry, with many isolates causing up to 100% lethality in domestic pigs. ASF is endemic in most sub-Saharan countries in Africa and in Sardinia. Since 2007 ASF has spread from Georgia in the Caucasus, to the Russian Federation and Eastern Europe including EU countries [1]. There is no vaccine for ASF and this limits disease control.

ASF is caused by a complex double-stranded DNA virus, African swine fever virus (ASFV), which encodes up to 167 genes [2,3]. Many genes encode proteins with roles in evasion of host defence's. Amongst these are proteins that inhibit type I interferon induction or responses including a TLR3 agonist, I329L, and members of MGF families 360 and 505/530 [4–6]. Levels of protection up to 100% against virulent virus challenge have been achieved by immunisation with attenuated ASFV. Deletion of multigene family members MGF 36-10L, 11L, 12L, 13L, 14L and 505/530 1R, 2R from the Pr4 isolate or MGF 360-12L, 13L, 14L and MGF 505/530 1R, 2R, 3R from the Georgia 2007 isolate [7] resulted in virus attenuation and induction of protection against challenge. We showed that deletion of these genes plus an additional deletion of MGF 505-3R and interruption of MGF 360-9L and MGF 505-4R from the Benin97/1 isolate (BeninΔMGF) also resulted in

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attenuation of the virulent Benin97/1 and induction of high levels of protection against virulent parental virus challenge [6].

In the current study we compared protection induced by intramuscular immunisation of pigs with the deletion mutant BeninΔMFG at different doses (10^2, 10^3, 10^4 $TCID_{50}$), together with intranasal immunisation at the 10^3 dose. The aim was to better define the safety and efficacy of this attenuated vaccine candidate and to understand its protective mechanisms. Since the BeninΔMGF strain is genotype I, the major genotype circulating in West and Central Africa and Sardinia, this strain may be a potential vaccine strain in these regions and others if cross-protection against other genotypes is confirmed as demonstrated for the OURT88/3 attenuated genotype I strain [8].

2. Materials and methods

2.1. Viruses, animals and experimental design

The preparation of viruses used, Benin97/1 and BeninΔMGF, were described previously [6,9]. Virus titres were shown as the amount of virus infecting 50% of the macrophages cultures ($TCID_{50}$/ml).

Experiments were conducted in SAPO4 high containment facilities at The Pirbright Institute and regulated by the Animals (Scientific Procedures) Act UK 1986. Large White and Landrace crossbred female pigs, 8–9 week-old (18–22 kg), from a high health status herd were used (Fig. 1A). Pigs were separated in groups of six and immunised with BeninΔMGF intramuscularly (IM) in the neck muscles with 1 ml containing 10^2 (group A), 10^3 (group B) and 10^4 (group C) $TCID_{50}$/ml. One group of six pigs (group D) was immunised intranasally (IN) with 2 ml (1 ml per nostril) containing 10^3 $TCID_{50}$ of BeninΔMGF. At day 21 post-immunisation (pi), pigs were boosted with the same dose and by the same route. After a further 18 days (day 39 pi/day 0 post-challenge, pc), all immunised pigs together with a control group (group F) containing three non-immunised pigs were challenged intramuscularly with 1 ml containing 10^4 $TCID_{50}$/ml of the parental virulent ASFV isolate Benin 97/1.

2.2. Quantitative PCR analysis of virus genome copy numbers

DNA was extracted from whole peripheral blood and analysed for ASFV genome detection by quantitative PCR (qPCR) [8,10].

2.3. Detection of immunoglobulins of isotype M and isotype G and cytokines in swine sera

Serum samples from immunised pigs were analysed using two newly developed ELISA assays (Ingenasa, Madrid; brief protocol described in Fig. 4) based on the semi-purified VP72 protein in order to detect the presence of IgM (capture assay), as an indicator of early infection, and IgG antibodies (indirect assay).

Porcine immunoregulatory cytokines (IFNγ, IL-1β and IL-10; R&D Systems) were assayed in serum samples following manufacturer's instructions.

3. Results

3.1. Percentages of protection and clinical signs after immunisation and challenge

The highest percentage of protection (5/6 pig protected; 83%) was achieved in group C (IM, 10^4 $TCID_{50}$) while group A (IM, 10^2 $TCID_{50}$) showed the lowest (3/6; 50%) (Fig. 1B).

Rectal temperatures and clinical signs were monitored as described [8]. A transient increase in temperature was observed in some immunised pigs for 1 or 2 days between days 4 and 6 pi (Fig. 2). In group A (Fig. 2A), 4/6 pigs (A1, A2, A3 and A5) had an increase in rectal temperature above 40.5 °C and in two of these (A1, A3) temperatures increased above 41 °C. In group B (Fig. 2B), only pig B6 displayed a transient increase in temperature post-immunisation (day 4 pi, 41.5 °C). In group C (Fig. 2C), 2 pigs (C4 and C5) displayed a slight transient increase in temperature at day 4 pi (40.7 and 40.6 °C respectively). Finally, in group D (Fig. 2D) none of the pigs showed clinical signs post-immunisation. No further clinical signs or increase in temperature were observed after immunisation or boost in any of pigs.

After challenge, non-immunised control pigs (group F) were euthanized at day 5 pc after reaching a moderate severity endpoint. Some immunised pigs in group A (A2, A4 and A6), group B (B2 and B6), group C (C5) and group D (D4 and D6) displayed clinical signs typical of acute ASF and were euthanized between days 4 and 12 pc. No clinical signs were observed in the remaining immunised pigs in any of the groups which were euthanized at day 19 (protected pigs in groups A and C) or 25 (protected pigs in groups B and D) post-challenge.

Statistical analysis showed very significant differences ($P < .0001$) among mean temperatures of pigs in group A, B and C (10^2, 10^3 and 10^4 $TCID_{50}$; IM) with respect to temperatures of pigs in group D (10^3 $TCID_{50}$; IN) at days 4 and 5 pi (Supplementary Fig. 1).

3.2. Levels of virus genome in blood after immunisation and challenge

The ASFV genome copy numbers in blood for individual pigs over the course of the experiment are shown (Fig. 3). Pig A6 did not have detectable levels of virus genome before challenge. The rest of the pigs immunised intramuscularly with different doses in group A, B, and C (Fig. 3A-C) had moderate levels of ASFV DNA (10^4–10^6 genome copies) in blood by day 4–7 pi, while only 4/6 pigs in group D immunised intranasally (Fig. 3D) had ASFV genome copies (10^2–10^4 genome copies) in their blood at this time. Detectable levels of virus genome before challenge were not observed in pig D2, and only detected a very low level at day 28 pi (day 7 after booster immunisation) in pig D1.

A similar trend was recognised in all groups, with genome copy numbers gradually decreasing from day 7 pi until challenge (day 39 pi/0 pc). In group A (Fig. 3A), pig A2 and A4 had lower levels of genome in blood than other pigs in this group (except A6 which had no detectable genome) and virus DNA was not detectable in blood samples of pig A4 from day 15 pi until after challenge. In group D (Fig. 3D), pig D6 also showed lower levels of genome in blood than other pigs except D1 and D2 which had no detectable DNA until after boost or challenge. Genome was not detected in pig D6 from day 15 pi until after challenge.

After challenge (Fig. 3A-D), viremia levels of protected pigs in all immunised groups did not display remarkable changes with respect to pre-challenge values (with values below 10^4 genome copies) and gradually decreased until the end of the experiment. At termination, viremia levels in protected pigs of group A (A1, A3 and A5) were between 10^2 and 10^3 genome copies. In group B only pig B5 displayed values above 10^2 genome copies. In group C ASFV genome copies were not detected in pigs C1 and C2, and only pig C3 showed values above 10^2 genome copies. Finally in group D, just one pig (D3) showed detectable levels of genome copies below 10^2 genome copies/mL at termination.

After challenge, non-protected pigs in all immunised groups showed an increase of virus genome copies that reached levels of 10^5 to 10^7 before euthanasia, except pig A2 which has lower levels of genome detected (10^3). All non-protected pigs were terminated with clinical and pathological signs of acute ASF similar to those

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