US Patent for Attenuated African swine fever virus vaccine Patent (Patent # 10,507,237 issued December 17, 2019)

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FIELD OF THE INVENTION

The present invention relates to attenuated African Swine Fever Viruses. The engineered viruses protect pigs against subsequent challenge with virulent virus. The present invention also relates to the use of such attenuated viruses to treat and/or prevent African Swine Fever. The invention further relates to intranasal administration of attenuated African Swine Fever Viruses.

BACKGROUND TO THE INVENTION

African Swine Fever (ASF)

African swine fever is a devastating haemorrhagic disease of domestic pigs caused by a double-stranded DNA virus, African swine fever virus (ASFV). ASFV is the only member of the Asfarviridae family and replicates predominantly in the cytoplasm of cells. Virulent strains of ASFV can kill domestic pigs within about 5-14 days of infection with a mortality rate approaching 100%.

ASFV can infect and replicate in warthogs (*Phacochoerus* sp.), bushpigs (*Potamocherus* sp.) and soft ticks of the *Ornithodoros* species, but in these species few if any clinical signs are observed

and long term persistent infections can be established. The disease is currently endemic in many sub-Saharan countries and in Europe in Sardinia. Following its introduction to Georgia in the Trans Caucasus region in 2007, ASFV has spread extensively through neighbouring countries including the Russian Federation. In 2012 the first outbreak was reported in Ukraine and in 2013 the first outbreaks in Belarus. In 2014 further outbreaks were reported in pigs in Ukraine and detection in wild boar in Lithuania and Poland.

There is currently no treatment for ASF. Prevention in countries outside Africa has been attempted on a national basis by restrictions on incoming pigs and pork products, compulsory boiling of waste animal products under license before feeding to pigs and the application of a slaughter policy when the disease is diagnosed. Prevention in Africa is based on measures to keep warthogs and materials contaminated by warthogs away from the herd.

To date, no effective attenuated or inactivated vaccines have been developed.

There is thus a need for improved measures to control ASFV infection and prevent spread of the disease.

African Swine Fever Virus (ASFV)

The ASFV genome encodes five multigene families (MGF 100, MGF 110, MGF 360 and MGF 505/530) located within the left hand 35 kb or right hand 15 kb terminal variable regions. The MGFs constitute between 17 and 25% of the total coding capacity of the ASFV genome. They lack similarity to other known genes. Although the function of individual MGF genes is unknown, it has been shown the MGF 360 and 505 families encode genes essential for host range function that involves promotion of infected-cell survival and suppression of type I interferon response.

The OURT88/3 Isolate

OURT88/3 is a non-pathogenic isolate of ASFV from Portugal. Previous infection with ASFV OURT88/3 has been shown to confer protection against challenge with related virulent viruses (Boinas et al (2004) J Gen Virol 85:2177-2187; Oura et al (2005) J. Gen. Virol. 86:2445-2450).

It has been demonstrated that CD8+ T cells are required for the protection induced by the OURT88/3 strain, since antibody mediated depletion of CD8+ T cells abrogates protection (Oura et al., 2005, as above).

Studies were carried out using the NIH inbred pig lines cc and dd. In these studies, a control group of 3 dd pigs and a control group of 6 dd pigs were immunised with OURT88/3. In the first of these experiments, following OURT88/3 immunisation one dd pig developed a transient low viremia of log 10 2-3 TCID 50/ml, but no fever or clinical signs of disease. It was observed that 3 of 6 of the cc inbred pigs immunised with OURT88/3 were not protected following lethal challenge with OURT88/3 isolate, whereas protective responses were induced in all dd pigs. These results indicate that the genetic background of the pig influences the response to OURT88/3 inoculation.

In subsequent experiments 5 dd and 5 cc pigs were immunised with OURT88/3 and challenged with OURT88/1 (Takamatsu et al., 2003 unpublished results). This confirmed that protective responses were induced in all dd pigs, but not induced in all cc pigs following immunisation with OURT88/3 and that adverse reactions including transient pyrexia, joint swelling and lameness were induced in some of the cc pigs.

Subsequent experiments were carried out in France using the

Anses herd of SPF pigs. In these pigs, similar to observations with cc pigs, some pigs developed adverse reactions including transient fever, joint swelling and lameness following immunisation with OURT88/3 (unpublished results).

Although OURT88/3 has been shown to induce a protective immune response in certain animals, this effect does not appear to be universal. Immunisation with OURT88/3 appears to be ineffective in protecting some pigs from subsequent challenge. It is also associated with the induction of adverse immune responses, such as joint swelling, in some pigs.

There is therefore a need for alternative ASFV vaccine candidates with improved efficacy and safety profiles.

DESCRIPTION OF THE FIGURES

FIG. 1—Schematic diagram showing the generation of recombinant virus BeninΔMGF with the deletion of five MGF 360 genes 10L, 11L, 12L, 13L, 14L and the deletion of three MGF 505 genes 1R, 2R, 3R. Recombinant virus BeninΔMGF was created by the homologous recombination between the MGF 360 gene 9L and the MGF 505 4R gene on the wild type Benin 97/1 genome and the transfer vector plasmid pΔMGFGUS resulting in the deletion of the eight MGF genes and the insertion of the GUS marker gene.

FIG. 2—Analysis of genomic viral DNA gene deletions and insertions by PCR. Viral DNA was extracted from wild type Benin 97/1 and the recombinant virus Benin ΔMGF. Specific fragments were amplified by PCR and the products were analysed on a 1% agarose TAE gel. The following primer sets were used in lane 1 (BeninD8F and BeninD8R), lanes 2 and 3 (BeninD8F and RGUS), lanes 4 and 5 (BeninD8INTF and BeninD8INT). Lane 0 contains a DNA ladder.

FIG. 3—DNA sequence analysis of recombinant virus BeninΔMGF at the deletion/insertion site compared to the published sequence of wild type Benin 97/1. Viral gDNA was isolated from BeninΔMGF infected cells and the left flank of the deletion/insertion was sequenced with primer 9LF and the right flank sequenced with primer 4RR. The left flank sequence of BeninΔMGF shows the insertion of sequences vp72 promoter, loxP and 5′ GUS gene and the deletion of eight MGF genes and deletion of the first five nucleotides of the MGF 360 9L gene including the ATG start codon. The right flank sequence of BeninΔMGF shows the insertion of the 3′ GUS gene and the deletion of the first seven nucleotides of the MGF 505 4R gene, including the ATG start codon.

FIG. 4—Replication kinetics of Benin 97/1 and recombinant BeninΔMGF viruses. Pig bone marrow macrophages were infected at high multiplicity of infection with parental BeninΔ97/1 or recombinant BeninΔMGF viruses. At various hours post-infection, as indicated on the x-axis, total virus was harvested and infectious virus titrated on 96 well plates by analysis of haemadsorption on cultures of pig bone marrow macrophages. The virus titre (HAD_{50}/ml) is the mean of three individual observations.

FIG. 5—Clinical scores post first inoculation. Clinical scores (y-axis) of individual pigs at different days (x-axis) post inoculation. Pigs 16, 18, 19 and 20 inoculated with 10^4 $TCID_{50}$ OURT88/3 virus. Pigs 21, 22, 23 inoculated with 10^2 HAD_{50} BeninΔMGFA2 virus. Pigs 24 and 25 inoculated with 10^2 HAD_{50} BeninΔMGFA1 virus. Clinical scoring system as designed by King et al 2011.

FIG. 6—Temperatures post first inoculation. Temperatures (y-axis) of individual pigs at different days (x-axis) post inoculation. Pigs 16, 18, 19 and 20 inoculated with OURT88/3 virus. Pigs 21, 22, 23

inoculated with BeninΔMGFA2 virus. Pigs 24 and 25 inoculated with BeninΔMGFA1 virus.

FIG. 7—IFN-γ ELISPOT assays. Peripheral blood mononuclear cells collected day 20 post first inoculation were stimulated ex vivo with either medium alone, OURT88/3 or BeninΔMGF. Results are shown as IFN-γ production per 10^6 lymphocytes (y-axis) and pig number (x-axis). Spots per 10^6 cells produced by PBMC purified on day 20 post inoculation with OUR T88/3 (pigs 16 to 20) or BeninΔMGF (pigs 21 to 25) in a porcine IFNγ ELIspot. Error bars represent standard deviation from the mean of duplicate wells. Dilution 1 contained twice as many cells as dilution 2.

FIG. 8—Clinical scores post boost inoculation. Clinical scores (y-axis) of individual pigs at different days (x-axis) post boost inoculation. Pigs 16, 18, 19 and 20 boost inoculated with 10^4 $TCID_{50}$ OURT88/3 virus. Pigs 21, 22, 23 boost inoculated with 10^4 HAD_{50} BeninΔMGFA2 virus. Pigs 24 and 25 boost inoculated with 10^4 $TCID_{50}$ BeninΔMGFA1 virus.

FIG. 9—Temperatures post boost inoculation. Temperatures (y-axis) of individual pigs at different days (x-axis) post inoculation. Pigs 16, 18, 19 and 20 inoculated with 10^4 $TCID_{50}$ OURT8813 virus. Pigs 21, 22, 23 inoculated with 10^4 HAD_{50} BeninΔMGFA2 virus. Pigs 24 and 25 inoculated with 10^4 HAD_{50} BeninΔMGFA1 virus.

FIG. 10—Clinical scores of pigs after challenge with Benin 97/1. Pigs from Groups 1 (pigs 21, 22, 23), Group 2 (pigs 24, 25), Group 3 (pigs 16, 18, 19, 20) and an unvaccinated Group 4 (pigs 26, 27, 28) were challenged with 10^4 HAD_{50} Benin 97/1 virus and the

clinical scores (y-axis) recorded at different days (x-axis) post challenge.

FIG. 11—Temperatures of pigs after challenge with Benin 97/1. Pigs from Groups 1 (pigs 21, 22, 23), Group 2 (pigs 24, 25), Group 3 (pigs 16, 18, 19, 20) and an unvaccinated Group 4 (pigs 26, 27, 28) were challenged with 10^4 HAD_{50} Benin 97/1 virus and the temperatures (y-axis) recorded at different days (x-axis) post challenge.

FIG. 12—Results of survival rates for all four groups of pigs. The y-axis shows the percentage of pigs which survived following initial inoculation (Day 0), boost inoculation (Day 25) and challenge (Day 46) with virulent virus Benin 97/1. Groups 1 and 2 ★, Group 3 ●, Group 4 □.

FIG. 13—IFN-γ ELISPOT assays. Peripheral blood mononuclear cells or spleen homogenate cells isolated on day 63 post first inoculation were stimulated ex vivo with either medium alone or Benin 97/1 virus. Results are shown as IFN-γ production per 10^6 lymphocytes (y-axis) and pig number (x-axis).

FIG. 14—Percentage of circulating total lymphocytes in peripheral blood samples (as compared with day 0) collected at different days post infection (x-axis) from pigs infected with OURT88/3 (pigs 16 to 20) or BeninΔMGF (pigs 21 to 25).

FIG. 15—Percentage of circulating CD4+ cells in peripheral blood samples (as compared with day 0) collected at different days post infection (x-axis) from pigs infected with OURT88/3 (pigs 16 to 20) or BeninΔMGF (pigs 21 to 25).

FIG. 16—Percentage of circulating CD8+ total cells in peripheral blood samples (as compared with day 0) collected at different days

post infection (x-axis) from pigs infected with OURT88/3 (pigs 16 to 20) or BeninΔMGF (pigs 21 to 25).

FIG. 17—Percentage of circulating CD8+CD4−γδTCR− (CD8 only) cells in peripheral blood samples (as compared with day 0) collected at different days post infection (x-axis) from pigs infected with OURT88/3 (pigs 16 to 20) or BeninΔMGF (pigs 21 to 25).

FIG. 18—Percentage of circulating CD8+/CD3− cells in peripheral blood samples (as compared with day 0) collected at different days post infection (x-axis) from pigs infected with OURT88/3 (pigs 16 to 20) or BeninΔMGF (pigs 21 to 25).

FIG. 19—Percentage of circulating gamma delta cells in peripheral blood samples (as compared with day 0) collected at different days post infection (x-axis) from pigs infected with OURT88/3 (pigs 16 to 20) or BeninΔMGF (pigs 21 to 25).

FIG. 20—Percentage of circulating gamma delta/CD8+ cells in peripheral blood samples (as compared with day 0) collected at different days post infection (x-axis) from pigs infected with OURT88/3 (pigs 16 to 20) or BeninΔMGF (pigs 21 to 25).

FIG. 21—Temperatures of pigs in Example 6 Groups A, B, C, D, E, F on different days post-immunisation and challenge and of control group G after challenge.

FIG. 22—Mean temperatures of all pigs in groups A to F of Example 6 after immunisation and challenge are shown in panel A. Panel B shows mean temperatures of surviving pigs in groups A to F and panel C temperatures of non-survivors.

FIG. 23—Clinical scores of pigs in Example 6 Groups A, B, C, D, E, F on different days post-immunisation and challenge and of control group G after challenge.

FIG. 24—Mean clinical scores of all pigs in Example 6 groups A to F after immunisation and challenge are shown in panel A. Panel B shows mean temperatures of surviving pigs in groups A to F and panel C temperatures of non-survivors.

FIG. 25—Scoring of lesions observed at necroscopy for different groups of pigs in Example 6. Mean scores for numbers of lesions observed in different groups from A to F are shown. Panel A shows scores of macroscopic lesions, Panel B of pulmonary lesions and Panel C of skin and musculoskeletal lesions.

FIG. 26—IFN-β mRNA induction by different ASFV isolates in primary porcine alveolar macrophages

FIG. 27—Shaded in grey is the sequence of the DP148R gene which was deleted from the ASFV genome. The start and stop codons are shown in bold. Sequences used to amplify the left and right flanking regions are underlined. The sequences amplified are between these primers.

FIG. 28—Growth curves of Benin97/1, BeninΔDP148R and BeninΔMGF in porcine alveolar macrophages.

FIG. 29—Temperatures of pigs immunised with BeninΔMGF at HAD_{50} doses of 10^2 (A), 10^3 (B) and 10^4 (C) using the IM route, 10^3 HAD_{50} using the intranasal route (D) and BeninΔDP148R at 10^3 HAD_{50} using IM route (E), on different days post-immunisation and challenge and of control group (F) after challenge.

FIG. 30—Clinical scores of pigs in groups A to E as described for FIG. 29 on different days post-immunisation and challenge and of control group F after challenge.

FIG. 31—Mean temperatures of all pigs in groups A to E as described for FIG. 29 after immunisation and challenge.

FIG. 32—Mean clinical scores of all pigs in groups A to E as described for FIG. 29 after immunisation and challenge

SUMMARY OF ASPECTS OF THE INVENTION

The present inventors have surprisingly found that deletion of five multi-gene family (MGF) 360 genes 10L, 11L, 12L, 13L, 14L and three MGF 505 genes 1R, 2R, 3R from the left hand end of the ASF virus genome and interruption of two additional genes (MGF360 9L and MGF 505 4R) resulted in attenuation of a virulent virus and induction of 100% protection against challenge with parental ASFV virulent virus.

Thus, in a first aspect; the present invention provides an attenuated African Swine Fever (ASF) virus which lacks a functional version of the following genes:

- multigene-family 360 genes 9L, 10L, 11L, 12L, 13L and 14L; and
- multigene-family 505 genes 1R, 2R, 3R and 4R.

The following genes may be at least partially (i.e. partially or completely) deleted:

- multigene-family 360 genes 10L, 11L, 12L, 13L and 14L; and
- multigene-family 505 genes 1R, 2R and 3R.

The following genes may be interrupted:

- multigene-family 360 gene 9L; and
- multigene-family 505 gene 4R.

The inventors have also surprisingly found that deletion of only one gene, namely the DP148R gene, from a region close to the right end of the virulent Benin97/1 genome, resulted in attenuation of a virulent virus and does not reduce virus replication in macrophages. A group of 5 pigs immunised with BeninΔDP148R intramuscularly