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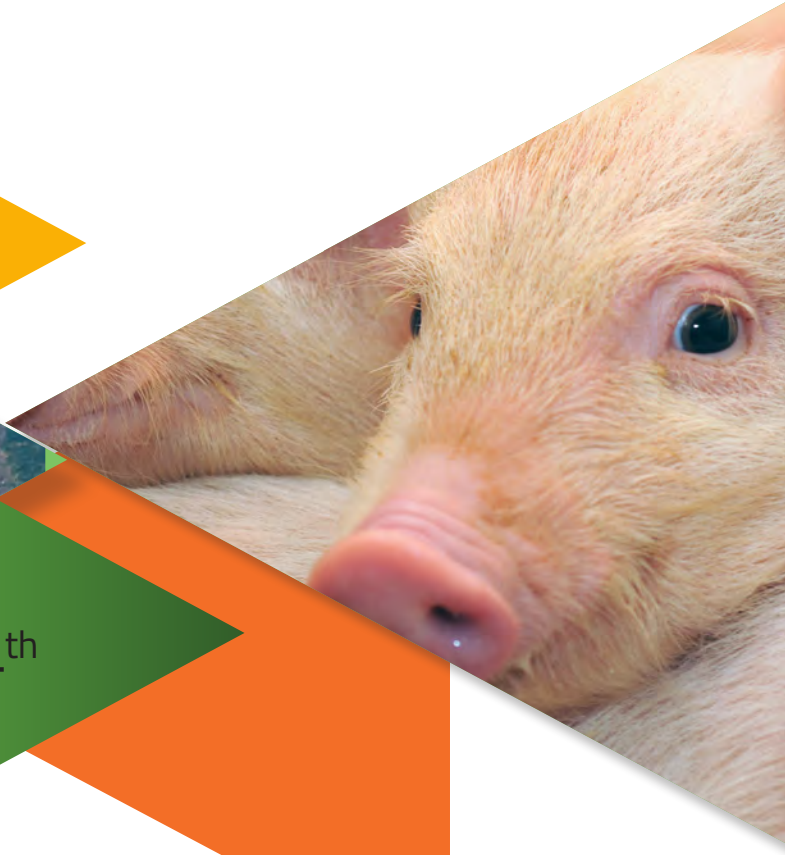

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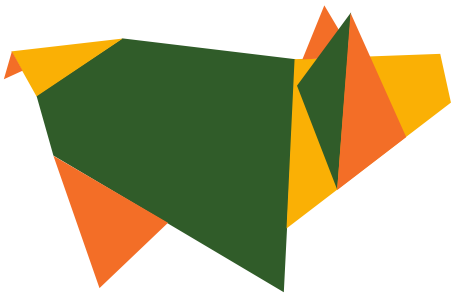

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Development of recombinant live attenuated vaccine candidates in ASF

Manuel V. Borca

Lead Scientist, DVM, PhD. Plum Island Animal Disease Center (PIADC),

Agricultural Research Service (ARS), U.S. Department of Agriculture (USDA)

Greenport, NY 11944, USA

Correspondence: manuel.borca@usda.gov

African swine fever (ASF) is a devastating disease of swine present in more than twenty sub-Saharan African countries and currently being considered a pandemic affecting countries in a large contiguous geographical area from Central Europe to East and Southeast Asia. In the last months, the disease was also detected in Dominican Republic and Haiti, after more than fifty years of being absent from the Western hemisphere. This situation is causing significant economic losses in the pig industry and a shortage in food availability in the affected countries. The causative agent of this epidemiological situation, African swine fever virus (ASFV) strain Georgia 2007 (ASFV-G), is a highly virulent strain that belongs to the ASFV genotype II group.

ASFV is a large and structurally complex virus, presenting a double envelope and harboring a large (180-190 kilobase pairs) double-stranded DNA genome, encoding approximately 150-160 genes. The role of most of these genes remain unknown or just have been predicted by functional genomics. Actually, just a few virus proteins have been experimentally studied to determine their function.

Currently, there is no commercial vaccines available to prevent ASF, so control of the disease is basically achieved by culling the affected animals. The current ASF pandemic has energized the research in the development of experimental vaccines to improve the epidemiological management of the disease. Among the experimental vaccines, those based in the use of attenuated strains constitutes the most successful candidates. Although approaches to develop attenuated ASFV strains have considered the use of naturally attenuated isolates as well as those obtained by adaptation to grow in cell cultures, vaccine candidates rationally designed and developed by genetic manipulation are the very promising alternatives. Attenuation of virulent viruses have been achieved by deleting ASFV genes associated with virulence in swine through genetic manipulation. Using this approach several experimental recombinant vaccine candidates have been developed which efficiently protect pigs against the challenge with the ASFV-G isolate or its field isolate derivatives. Therefore, discover of virus genes important in the process of disease production is a critical first step in the design of recombinant LAVs to prevent ASFV infection and disease. Discovering ASFV genes involved in virus virulence is a laborious and time demanding process based, essentially, on a systematic experimental approach. At the moment, only fifteen ASFV genes or a group of genes, have been identified as determinant of virulence since their individual deletion produced a significant decreased of virulence of the corresponding parental virulent strain. Nine of these determinants of virulence have been shown to produce attenuation in the ASFV-G, or any of its derivatives, and those have been demonstrated that can be used to develop an attenuated strain able to induce protection against the challenge with the field isolate responsible for the current pandemic affecting Eurasia. The deletion of even highly conserved virus genes have been shown to have different consequences in terms of virus virulence depending on the virus strain considered. These factors make the identification and characterization of novel genetic determinants of virulence an essential issue for the rational production of the next generation live attenuated vaccine candidates to protect swine against the current pandemic strain ASFV.

Here, we will review the different attempts performed in our laboratory in the development of recombinant live attenuated vaccine candidates inducing protection against the Georgia isolate. The design and development of these recombinant viruses will be analyzed and discussed in some detail.

Our first attempt to develop a live attenuated strain based in the ASFV-G isolate was the deletion of the 9GL gene (O'Donnell et al., 2015b). Deletion of 9GL gene produced attenuation of the ASFV-G virulent phenotype when used at relatively low doses (less than 10^3 HAD_{50}) but showed residual virulence a higher dose. Regardless its residual

virulence, when used at sublethal dose (at 10^3 HAD_{50} or less), ASFV-G-Δ9GL induced an efficacious protection against the IM challenge with the ASFV-G isolate both, at 21 and 28 days pv. ASFV-G-Δ9GL was one of the first recombinant attenuated viruses reported to induce protection against the epidemiologically important Georgia isolate.

To increase safety profile to ASFV-G-Δ9GL we combined the deletion of the 9GL gene with the additional deletion of the UK gene. A virus harboring deletions of both genes, named ASFV-G-Δ9GL/ΔUK, presented a drastically more attenuated phenotype than the parental ASFV-G-Δ9GL (O'Donnell et al., 2017). Animals inoculated with up to 10^6 HAD_{50} of ASFV-G-Δ9GL remained clinically normal and were effectively protected against the IM challenge with 10^3 HAD_{50} of Georgia isolate.

Attenuation of virulent ASFV was also achieved by deleting 6 genes belonging to the MGF360 and MGF505 groups from the genome of the highly virulent ASFV-G isolate (O'Donnell et al., 2015). Pigs inoculated IM with up to 10^4 HAD_{50} of the resulting recombinant virus, ASFV-G-ΔMGF, remained healthy, without signs of the disease and, when they were challenged with virulent parental ASFV-G strain, no signs of the disease were observed although a proportion of these animals harbored the challenge virus.

Another single gene deletion recombinant virus with potential vaccine capability was developed by deleting the I177L gene from the genome of the virulent ASFV-G (Borca et al., 2020). The recombinant virus lacking this gene, ASFV-G-ΔI177L, present a drastic decrease in virulence when inoculated in pigs. Even those receiving up to 10^6 HAD_{50} IM remained clinically normal and were completely protected against the challenge IM with ASFV-G virus at 28 days pv, even those receiving as little as 10^2 HAD_{50} of ASFV-G-ΔI177L. Animals vaccinated with doses of 10^4 HAD_{50} or higher of ASFV-G-ΔI177L developed sterile immunity against the challenge virus.

Interestingly, efficacy of ASFV-G-ΔI177L was also tested using as challenge virus a highly virulent field isolate from Vietnam, TTKN/ASFV/DN/2019 (Hanh et al., 2021). ASFV-G-ΔI177L induce protection against TTKN/ASFV/DN/2019 challenge with a similar efficacy than against the Georgia2007 strain in experiments conducted in parallel using pigs with both, European (Yorkshire/Landrace crossbread) as well as Vietnamese genetic background (Mong Cai breed).

ASFV-G-ΔI177L was also tested when administered by oronasal route (Borca et al., 2021), exploring its potential use as an oral vaccine. Animals oronasally inoculated with ASFV-G-ΔI177L and IM challenged 28 days later with the virulent ASFV-G isolate were protected, not showing clinical signs associated with ASF.

A modification of ASFV-G-ΔI177L has been obtained by adapting the virus to grow in an established swine cell line, as a mean to facilitate its production at industrial scale (Borca et a., 2021b). The adapted virus, ASFV-G-ΔI177L/ΔLVR, showed a large and stable deletion in the left variable region of its genome. Challenge studies performed in domestic pigs showed that ASFV-G-ΔI177L/ΔLVR maintained the same level of attenuation, immunogenic characteristics, and protective efficacy as ASFV-G-ΔI177L.

Finally, deletion of the ASFV gene A137R has also shown to attenuate ASFV-G isolate (Gladue et al., 2021). Removal of the gene from the genome of the highly virulent parental virus produced a virus, named ASFV-G-ΔA137R which, when IM inoculated in pigs (10^2 HAD_{50}) showed a strong attenuation. Interestingly, all ASFV-G-ΔA137R inoculated animals were protected when IM challenged with the virulent parental strain ASFV-G without showing evidence of replication of challenge virus.

An update of the status of development of these vaccine candidate strains will be presented along with several potential recombinant vaccine strains developed in other laboratories.

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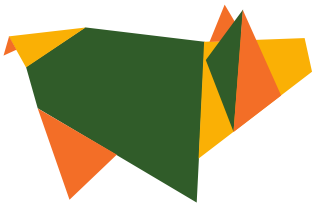

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