Vaccines against African Swine Fever: Yes, we can!

Why is African Swine Fever worrying pig production? One – the disease continues to cause problems in Russia, two – there is no vaccine. Yet, that is. Spanish researchers recently found that protective T-cells may change this.

African Swine Fever virus (ASFV) is the causal agent of a serious disease which affects domestic pigs and causes important economic losses to the affected countries. ASFV is the only DNA arbovirus (ar thropod- bo rne- virus) known, and its perpetuation and transmission in Africa, where the virus remains endemic, involves a sylvatic cycle (i.e. occurring in the wild) between ticks of the Ornithodoros genus and wild pigs that are resistant to the disease, becoming a continuous source of virus, therefore complicating its eradication. As an example of its devastating consequences, ASFV has reduced half the total number of pigs in Madagascar since its introduction in 1998.

Control measures of the disease applied in Europe, based on an efficient diagnosis and the sacrifice of the affected animals, have not been effective in developing countries, mainly due to economic reasons and aggravated by the close and continuous presence of the natural reservoirs (wild pigs and ticks); making correct implementation impossible.

The development of vaccines against ASFV has been almost entirely neglected, mainly due to the technical difficulties involved in its development and to the fact that ASF was considered an ‘exotic’ disease in developed countries (at least since its eradication from Spain and Portugal in the mid-1990’s). The situation has dramatically changed with the recent entrance of the virus in Georgia from eastern Africa.

The armed conflict between Georgia and Russia favoured the initial spread of the virus to neighbouring regions, including Armenia, Azerbaijan and many Russian regions, some of them located thousands of kilometres away from the original point of ASFV first entry. The situation worsened with the continuous declaration of outbreaks, also with sporadic cases reported in Ukraine and Iran, threatening the rest of the world, including

the largest pork consumer and producer in the world, China.
As demonstrated for many diseases, having an efficient vaccine against ASFV will be essential in controlling the disease in endemic regions and the dissemination of the disease around the rest of the world.

Availability of ASF vaccines

Why are ASF vaccines not available? To our understanding, there are two main reasons explaining the lack of available vaccines against ASF. First of all, the high complexity of the virus has complicated this issue. ASFV is a large double stranded DNA virus that encodes more than 150 different proteins, with the ASFV particle containing at least 50 proteins arranged in several layers. In comparison, Porcine Circovirus type 2 particles are composed by one only polypeptide, the cap protein.

Additionally, ASFV encodes multiple virulence factors allowing its replication in porcine macrophages and the concomitant evasion from the host immune response, therefore complicating the development of efficient antiviral strategies.
Second, and as mentioned above, little effort has been made to obtain a safe and efficient vaccine against ASF, leading to the probable false perception that this was an impossible task to pursue.

Thus, compared with the deep knowledge that we have today about different aspects of the complex biology of ASFV, efforts made to obtain an efficient vaccine have usually been scarce and most of them were performed more than ten years ago. Three different strategies have been followed in the past:

1. Inactivated vaccines

Inactivated vaccines of ASFV were capable of inducing antibody responses that, however, did not translate to efficient protection.

2. Attenuated strains

In clear contrast, immunisation of pigs with classically attenuated strains of ASFV (natural isolates or tissue culture adapted viruses) induced very solid protection against the homologous viral challenge. Safety issues made the application of these live-attenuated viruses as vaccines impossible, they have however provided us with the most useful data existing today about immune parameters involved in protection. Thus, both antibodies and cytotoxic specific CD8+ T-cells were demonstrated to play important roles in the protection afforded by live-attenuated vaccines.

2a. Antibodies and T-cells

Neither antibodies nor T-cells seemed to be able, by themselves, to confer complete and sterilising protection, indicating that an ideal vaccine against ASFV should be able to confer both kinds of immune responses. While specific antibodies could more efficiently

neutralise and/ or inhibit the virus particles found in suspension (blood and other corporal fluids); CD8+ T-cells (cytotoxic T-cells) would be able to recognise and destroy ASFV infected cells.

2b. Deletion of genes

Deletion of specific virulence genes by homologous recombination allowed the construction of live attenuated ASFV viruses, albeit several genes should be simultaneously deleted in order to comply with the minimum security issues requested for any commercial vaccine. This alternative will require further research on ASFV pathogenesis, allowing a more rational selection of the virulence factors to be eliminated from the virus in order to obtain the safest and most efficient recombinant vaccine.

2c. Replication deficient strains

An attractive alternative to the classical deletion of genes is the use of inducible viruses as vaccines, a strategy that is currently being explored by the group of Dr Salas, at the CBMSO in Madrid, Spain. The idea behind this technology is to develop replication deficient ASFV vaccine strains. So far, in vitro experiments have demonstrated that these cells produce, under restrictive conditions, 'empty' viral particles that lack the inner contents.

Such virus-like particles possess all the external domains (inner envelope and capsid) and can exit efficiently from the infected cell, but they are not infectious.

This strategy should comply with all the requisites for an ideal vaccine against ASFV. Again, further research is needed.

3. Subunit vaccines

In terms of safety, subunit vaccines should be the preferred choice.However, the complexity of ASFV influences the task of selecting the optimal antigens to be included in a vaccine. Several reports exist describing antigenic viral proteins, but little has been reported about their protective efficacy. Immunisation with peptide 'cocktails' showed a slight delay in the mortality found after experimental infection, while vaccination with entire viral proteins yielded contradictory results. Work done in the mid-1990's described the protective potential of three ASFV structural proteins: p54, p30 and hemagglutinin (HA), when expressed in a baculovirus system and administered without further purification.

Especially the results for subunit vaccines, provided ingredients for further studies. More than ten years after the first description of the protective capabilities of p54, p30 and HA, researchers at the Centre de Recerca en Sanitat Animal (CReSA) decided to extend these studies to the field of DNA immunisation. This strategy is considered both simple and affordable – and manages to induce both antibody as well as T-cell responses, necessary to obtain a good protection, see . The scientists were able to obtain conclusions that they believe will facilitate obtaining a more rational, safe and efficient

vaccine against ASF in the near future.

1. Targeting antigens

Targeting antigens to antigen professional cells (mainly macrophages and dendritic cells) exponentially enhanced the immune response induced in pigs; however, the DNA vaccine was not able to confer protection against lethal viral challenge. Indeed, a viraemia exacerbation was observed in each of the pigs that received the vaccine, this correlated with the presence of non-neutralising antibodies. The implications of these discoveries drove the scientists to 'tailor-made' vaccines, designed to enhance the CD8 T-cell responses, while avoiding the induction of non-neutralising antibodies. The 'trick' that was used to design such vaccines was based on the fact that CD8 T-cells are only capable of recognising very small protein fragments, i.e. peptides, on the surface of the cell. To force the intracellular degradation of the vaccine encoded proteins, it was decided to label the viral antigens with ubiquitin, a small and 'ubiquitous' cellular protein that marks proteins for rapid intracellular degradation in the proteasome; cytoplasmic proteolytic machinery that degrades the proteins into very small peptides, which are susceptible to being presented on the surface of the antigen presenting cell to the specific CD8 T-cells

2. Partial protection

Partial protection against ASFV lethal challenge was demonstrated after DNA immunisation of pigs with a plasmid encoding only three ASFV antigens: p54, p30 and the extracellular domain of the hemagglutinin (sHA), fused to ubiquitin. This plasmid was called pCMV-UbsHAPQ. As expected, the protection was afforded in the absence of antibodies, correlating with the expansion of CD8 T-cells that specifically recognised two peptides of nine aminoacids from the sHA, one of the three antigens encoded by the vaccine.Preliminary immunisation experiments with these two synthetic peptides confirmed their protective capabilities. The identification for the first time of specific protective CD8 T-cell epitopes not only confirmed the relevance of this kind of T-cell response in protection against ASFV but also opened the possibility of generating peptide-based vaccines using more potent expression vectors (see below).

3. BacMam

These results have been confirmed more recently by using an alternative vaccine delivery technology, named BacMam, a baculovirus vector that expresses the antigens of interest (in this case the sHA, p54, p30 from ASFV), under the control of a mammalian promoter. Immunisation of pigs with the recombinant BacMam-sHAPQ was able to protect pigs against sublethal challenge in the absence of specific antibodies. Protection again, correlated with the presence of a high number of ASFV-specific T-cells in their blood. These results definitively demonstrated the key role that T-cells play in protection against ASFV.

Efficacy improvement

Aiming to improve the efficacy of the vaccines, the CReSA scientists are currently extending their studies in two complementary directions. First, they are identifying additional protective determinants from within the ASFV-genome and second, they are also exploring alternative vaccination protocols aiming to optimise the immune responses induced, including the use of more powerful expression vectors, prime-boost regimes and the use of different adjuvants. In summary, results obtained at CReSA show the feasibility of obtaining safe and efficient vaccines against ASFV. Obtaining the optimal vaccine formulation is just a matter of time, investment and willingness.

References available on request.

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