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NEW TECHNOLOGIES FOR AFRICAN SWINE FEVER VACCINES

D2.5 Initial report on ASFV antigens predicted to induce cross-protective cellular or antibody responses

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1. Obtention of peripheral blood mononuclear cells (PBMC) from pigs immunised with genotype IX ASFV (Kenya1033)

To evaluate cellular responses to different ASFV antigens, PBMC obtained from immunised pigs are required. Before the beginning of the project, the Pirbright Institute (TPI) already had a biobank of frozen PBMC from 101 pigs immunised with a panel of Georgia/2007 deletion mutants (genotype II) of which, 79 survived the challenge with the parental Georgia/2007 virulent isolate. At the International Livestock Research Institute (ILRI), this type of sample was not readily available, and therefore an animal experiment was carried out. Briefly, eight 12-16 week old Duroc x Landrace x Large White pigs were randomly assigned to the immunised group (5 pigs) or to the control group (3 pigs). After 21 days of quarantine and acclimatisation, animals in the immunised group were inoculated via the intramuscular (IM) route with the attenuated ASFV-Kenya1033ΔMGF360.MGF505 deletion mutant (genotype-IX). Control pigs were mock-immunised with PBS by the same route. At 28 days post-immunisation (dpi), pigs in both groups were challenged with the parental Kenya1033 virulent isolate. As expected, animals in the control group developed clinical signs typical of acute ASF and were culled when they reached a pre-determined humane endpoint, at 5 days post-challenge (dpc). In the immunised group, 3 pigs survived challenge with Kenya1033; the other 2 pigs in the group reached their human endpoint at 5 dpc, in a similar manner to the control non-immunised pigs. Blood and serum samples were collected throughout the experiment. PBMC were purified from blood, by gradient centrifugation, at 27 dpi and at 49 dpi (protected pigs only).

2. Screening of ASFV peptide pools recognised by genotype IX immunised pigs

2.1 Quantification of number of IFNγ secreting cells by Elispot

The PBMC described in section 1 above, were used to quantify the number of IFNγ secreting cells in response to several ASFV antigens. This was done using a standard IFNγ ELISpot assay. Briefly, the cells were seeded in 96 well plates previously coated with a capture antibody against porcine IFNγ. Cells were then stimulated with 217 peptide pools covering 161 open reading frames (ORFs) from the Kenya1033 ASFV isolate. These 217 pools comprised 6,530 20-mer peptides, overlapping by 12 amino acids. Following overnight stimulation, the cells were removed, and the plates were probed with a detection antibody that also binds to porcine IFNγ. The detection antibody was then labelled with streptavidin linked to an enzyme. The addition of the enzyme substrate allowed the detection of “spots” which were then quantified and correspond to the number of IFNγ secreting cells. The results from this assay are shown in Figure 1. On the left are the non-protected pigs (PV086 and PV093) and on the right the pigs that survived challenge with Kenya1033 (PV089, PV091 and PV092). The graphs’ y-axis shows the number of spots per million of cells, and the x-axis shows the peptide pool used to stimulate the cells. The red bar crossing the y-axis represents the cutoff above which the response is considered positive. The cutoff was calculated for each individual pig, as 2 times the number of spots counted in cells treated with medium only.

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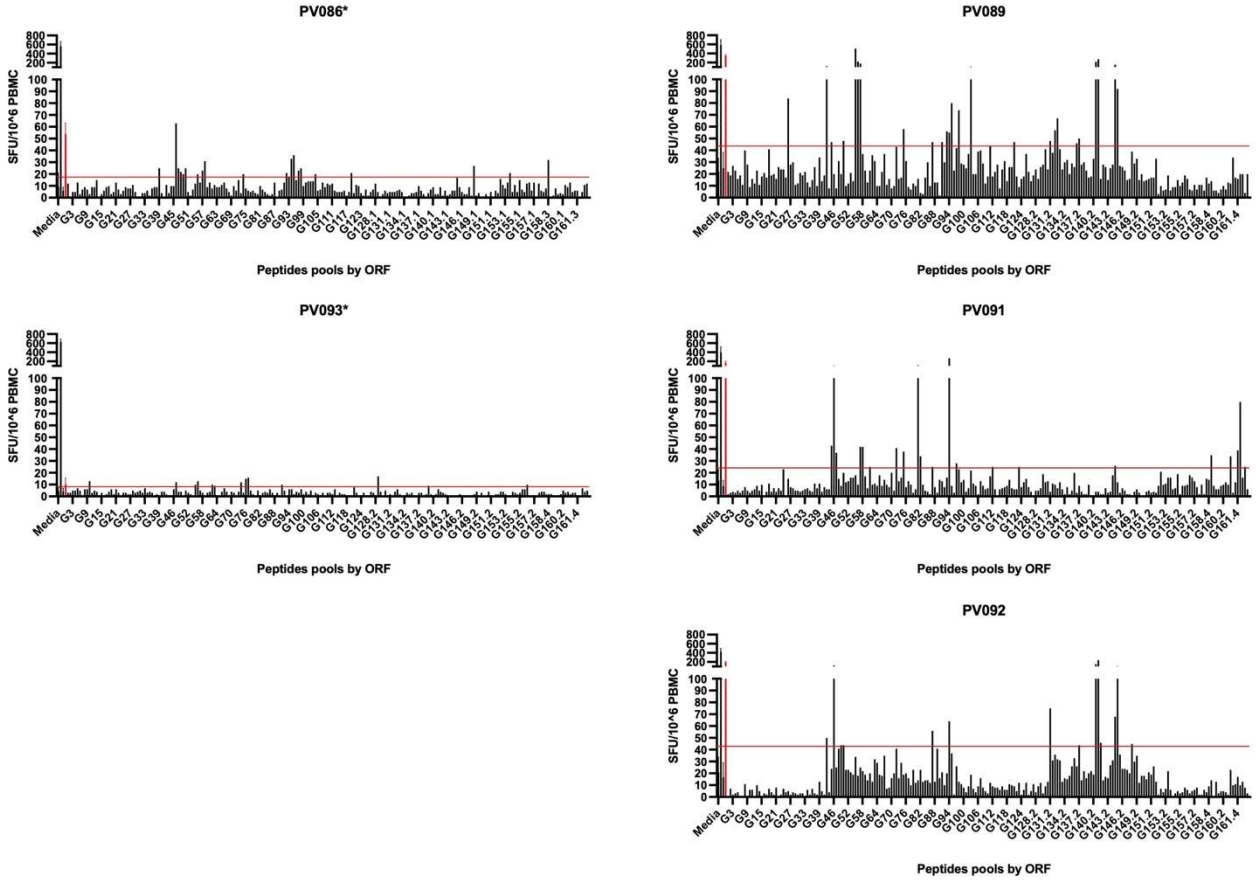

Figure 1. Number of IFNγ spot forming units (SFU) following stimulation of PBMC, from five individual pigs, with pools of ASFV peptides

2.2 Comparative analysis of control, vaccinated non-protected and vaccinated protected pigs

Following the identification of the ASFV antigens recognised by the 8 individual pigs (control and immunised pigs), a comparative analysis was done to highlight differences between the groups (Figure 2). Non-vaccinated pigs responded to stimulation with 9 ASFV antigens, and 4 of those antigens were also recognised by the vaccinated pigs. Of the 43 antigens recognised by the vaccinated pigs only, and therefore likely to represent a specific response to ASFV, only 18 were particular to the protected pigs (Figure 2, left chart). A more detailed analysis of the three protected pigs showed that only one antigen was recognised by all the animals (Figure 2, right chart). This data seems to indicate that the cellular responses against ASFV antigens are highly heterogenous.

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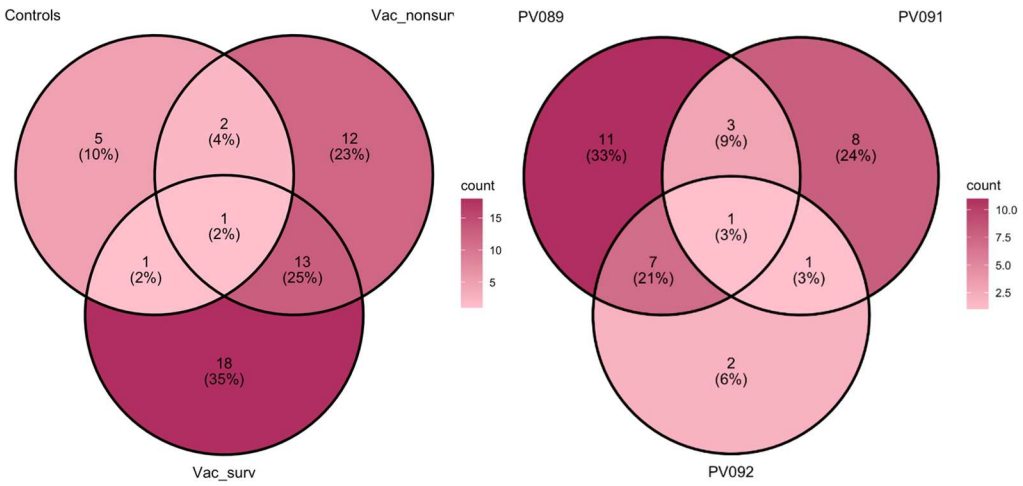

Figure 2. Left: overlapping chart comparing ASFV antigens recognised by control, vaccinated/non-protected and vaccinated/protected pigs. Right: overlapping chart comparing ASFV antigens recognised by the three surviving pigs (PV089, PV091 and PV092)

2.3 Identification of unique antigens recognised by protected pigs

As described above, protected pigs responded to a wide range of ASFV antigens. Table 1 shows the code of the ORF pools and the fold change in IFNγ SFU as compared to medium treated cells. This preliminary list will be further expanded by the results obtained at TPI using a library of peptides encoded by the Georgia/2007 (genotype II) isolate. This will allow the identification of those antigens commonly recognised by pigs protected against genotype IX or against genotype II.

Code for ORF pool	Range of fold-change
G26	3.78
G42	2.38-5.54
G44	2.12-3.21
G72	3.06
G75	2.61-2.84
G81	8.73
G82	2.54
G87	2.12-2.67
G103	5.18
G121	2.12
G131	2.16-3.57
G132	2.57
G133	3.02
G137	2.07-2.25
G141	7.29-12.52
G145	3.24-7.07
G159	2.61
G161	2.54-5.97

Table 1. ASFV antigens recognised by pigs that survived challenge with genotype IX ASFV

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3. Screening of sera for detection of haemadsorption (HAD) inhibiting antibodies

Eight different ASFV seroimmunotypes were described based on the ability of attenuated viruses to induce cross-protection against challenge with heterologous ASFV isolates. This correlated with the ability of sera from immunised pigs to inhibit HAD (doi: 10.3390/pathogens9040274). Therefore, we screened sera from 89 pigs immunised with genotype II Georgia/2007 deletion mutants, for the presence of HAD inhibiting antibodies. Briefly, purified pig bone marrow cells were infected at 1 x 10^3 HAD_{50}/ml with the homologous Georgia/2007 isolate. At 16 hours post infection, pre-challenge sera (2-fold serial dilutions starting from 1:2 up to 1:1024) were added to the cells. After 4 hours, the cells were washed, and diluted pig erythrocytes were added to the wells. Twenty-four hours later, plates were read for HAD inhibition activity. Unfortunately, none of the sera tested reduced HAD, which precluded the use of these sera against heterologous ASFV isolates.

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