VETBIONET

Veterinary Biocontained facility Network for excellence in animal infectiology research and experimentation

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Quantity of access provided over the duration of the project to FLI3

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This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

1. TNA Provided

Name of the TNA project	Name of TNA user	Organisation of TNA user	Country of TNA user	Installation from the RI	Start date	End date	Number of units of access provided
Pathogenesis and transmission of bat influenza viruses	Ghazi Kayali	Human Link	US	FLI3	Mar 2020	Apr 2020	EUR 15.265,54 (actual costs)
Characterization of the protection and responses elicited in pigs by a dendrimeric peptide (B2T) FMD vaccine including a new T cell epitope	Francisco Sobrino	Centro de Biología Molecular Severo Ochoa, UAM, Madrid	ES	FLI3	Sep 2022	Oct 2022	EUR 4.836,72 (actual costs)

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2. Final reports of each TNA provided

2.1 TNA 1: **Pathogenesis and transmission of bat influenza viruses**

It was recently discovered that bats are a natural reservoir for influenza A viruses. The TNA user has been conducting active surveillance for bat viruses in the Middle East and North Africa (MENA) since 2013. Since then, more than 2,300 bats from different bat species were sampled. Testing oral and rectal swabs for influenza A from around 600 Egyptian fruit bats revealed around 10% PCR detection rate. Of those, three samples were selected for isolation and all three were successfully cultured in embryonated chicken eggs and MDCK cells yielding the first wildtype isolates. Full genome sequence analysis indicated that the gene constellation is distinct from any previously known influenza genome. The HA and NA genes were around 70% similar to H9 and N2 subtypes. The other genes were around 80% similar to genes from H3, H7, and H13 viruses of North American and Eurasian origins. The pathogenesis of the virus in its natural reservoir was completely unknown at this time. Hence, the TNA user conducted a natural infection experiment using the bat isolate A/bat/Egypt/381OP/2017 (H9N2) at the FLI (access provider), where a colony of Egyptian fruit bats is kept. We infected a total of six bats intranasally, and on day 1 post infection, six naïve bats were introduced.

None of the inoculated or contact animals showed any obvious clinical signs of an infection, such as anorexia, depression or signs of a respiratory infection. We observed nasal shedding of low levels of viral RNA until day 9 post infection, but no replicating virus was detected. Nasal conchae and trachea samples of two inoculated animals sacrificed at 7 dpi tested positive for viral RNA. Nasal conchae from two of four inoculated animals sacrificed at day 21 were still positive for viral RNA of H9N2 bat flu. In addition to virus isolation and propagation, we observed that all animals directly inoculated with A/bat/Egypt/381OP/2017 (H9N2) and euthanized at 21 dpi displayed seroconversion.

We therefore conclude that the Egyptian fruit bat, although the challenge virus has been isolated from this species, only developed a transient subclinical infection.

2.2 TNA 2: **Characterization of the protection and responses elicited in pigs by a dendrimeric peptide (B2T) FMD vaccine including a new T cell epitope**

The identification of B and T FMDV dendrimeric peptides to be included in the formulation of vaccines to optimize protection is an important and timely topic for FMD vaccinology. In addition, deciphering the immune mechanisms mediating such protection is important for the development of new subunit vaccines against this important animal disease, as well as for their improvement in order to increase their immunogenicity and the protection conferred. A dendrimer peptide, termed B2T-3A, displaying two copies of the major FMDV antigenic B-cell site [VP1 (140-158)], covalently linked to a heterotypic T-cell epitope from the non-structural protein 3A [3A (21-35)], has been shown to protect pigs against viral challenge. The modular design of this dendrimer peptide allows modifications aimed at improving its immunogenicity, such as the replacement or expansion of the T-cell epitope moiety with an epitope from the FMDV 3D protein [3D (56-70)].

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The dendrimeric constructs analyzed in this experiment are marker subunit vaccines and have a reduced cost of synthesis, which make them promising FMD vaccine candidates. EU countries implement a non-vaccination, stamping out policy (i.e., slaughter of animals either infected or having been in contact with infected animals) for the control of FMD, which includes severe restrictions in the movement of animal and animal products in case of outbreaks. Vaccination is only allowed under emergency conditions, largely due to the problems associated with the serological distinction between infected and vaccinated animals. This entails a serious risk of disease reintroduction, significantly enhanced by the increase in global trade and the difficulties in monitoring farm product imports. The high primary (animal culling) and secondary (trade, travel, tourism restrictions) costs of recent outbreaks have fueled a strong debate on the possibility of vaccination in situations of disease reintroduction, and also stressed the relevance of research on safe, effective, marker vaccines with short development calendars allowing fast intervention in the event of an outbreak.

Three groups of 6 grower pigs were immunized with a single intramuscular dose of 2 mg of the B2T3D, B2T3A3D or B2T3A peptides emulsified with Montanide ISA 50V2. Two control groups of 3 pigs received a commercial FMD vaccine or PBS with adjuvant. After 21 days, they were challenged with FMDV O/PanAsia-2 by intradermal inoculation into the heel bulb of the right hind leg. The dendrimer peptides and the virus for challenge were provided by CISA. FLI provided the animals and the commercial type O vaccine. Viremia and virus excretion after challenge were quantified by RT-qPCR. The animals were monitored for FMD clinical signs such as elevated temperature or vesicles for 10 days. When dendrimer-vaccinated animals develop vesicles after challenge, the potential selection of escape variants was studied by sequencing the viral genome region corresponding to the peptides included in the dendrimer. Virus neutralization activity was determined in sera by a standard microneutralization test. Total antibodies against FMDV were examined by ELISA in serum samples collected at day 21. FMDV-specific IgG1, IgG2 (in sera), and IgA (in sera and nasal swabs) were measured using a modification of the indirect double antibody sandwich ELISA. Blood samples were used to obtain PBMC by density-gradient centrifugation. For ELISPOT-IFNγ and ICCS (IFNγ TNFα, CD8β), the cells were recalled in vitro with synthetic peptides and mitogens using frozen PBMCs.

The protection conferred and the B- and T-cell responses elicited in pigs by the dendrimer peptide B2T-3D including a new T cell epitope were analyzed and compared to the protection elicited by B2T-3A. The effect of combining two T cell epitopes (T-3A and T-3D) in a single molecule on the protective responses elicited by B2-T dendrimers was assessed. The results contribute to our understanding of the immunogenic potential of B2T-type dendrimers, which will be used to improve next-generation dendrimer peptide vaccines to control FMD in an important natural host of this virus: the pig.

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