COVRIN-D. 2.3.3 Establishment of virus-host interaction parameters

JIP COVRIN WP2

Responsible Partner: NVI

Contributing partners: Anses (P1), FLI (P10), APHA (P21), IZSAM (P28), WBVR (P31), NVI (P33)

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COVRIN-D. 2.3.3 ESTABLISHMENT OF VIRUS-HOST INTERACTION PARAMETERS

Title 1

1. Description of the task

The overall objective of task 2.3 in WP2 is to focus on developing animal models of SARS-CoV-2 infection and viral response/biomarker analysis from the host. The main two tasks performed under the 2.3.3 subtask are to i) participate in the studies that aim to decipher the virus-host interaction parameters and ii) summarize methodologies available to study the host immune responses generated in viral challenge studies in animal/cell culture models among partner institutes.

2. Description of the deliverable

2.1 Overview of studies initiated to decipher the virus-host interactions parameters in infection models

This work includes a collaborative study between FLI (P10) and NVI (P33) using samples from the screening of susceptible cell lines to SARS-COV2 strains performed at FLI (WP2-T2.2-ST2.2.1). Two wild boar-origin cell lines were highly susceptible and exhibited efficient virus spread in the cell cultures. In addition, RNA extracts from SARS-CoV-2 infected cells of high and lower susceptibility have been shared, and RNAseq analysis was performed at NVI (P33). The outcome of this study is expected to provide information on viral and host gene expression and other interaction parameters at the transcriptome level. The results will be published in collaboration between FLI and NVI.

2.2 Overview of the methods used to study host responses to SARS-COV2 infection using *in vivo* models.

The inquiry was sent to partner institutes to collect an overview of methods used to study host responses to SARS-COV2 infection using in vivo and In vitro models in COVRIN. The task was concluded in March 2023.

The main method of choice for host response monitoring was;

Serology	RBD antibody enzyme-linked immunosorbent assay (ELISA)
	Virus neutralization test (VNT)
Histopathology	Immunohistochemistry (anti-S or anti-NP antibody)
	In Situ Hybridisation (RNA probes targeting the S gene)
	Cytopathic effect (CPE)
Immunology	WBC count
	Cytokines measurement (ELISpot, Luminex multiplex analysis)
	Flow cytometry of tissue and blood samples (CD4/CD8 phenotyping)
	Whole transcriptome analysis (RNA sequencing)
	Targeted gene expression profiling (NanoString immunopanel, qPCR)
Physiological parameter	Body core temperature, locomotion activity, and heart rate by implanting data loggers, Daily water and energy expenditure

The main *in vivo* animal models established among partner institutes were,

- Ferret; Anses (P1); FLI (P10), APHA (P21)
- Syrian Gold hamster; Anses (P1), FLI (P10), WBVR (P31)
- Transgenic K18-ACE2 mice; FLI (P10), WBVR (P31), IZSAM (P28)

Histopathology techniques and serology methods were routinely used in the above animal models. Ferret models were mainly used for pathogenicity and the antibody responses generated against SARS-COV2 and were measured using ELISA. Golden Syrian Hamster and K18-ACE2 mouse models were used to study certain aspects of disease pathology and to perform vaccine and drug efficacy studies. In this model, innate and adaptive immune responses in different tissue compartments against SARS-CoV-2 variants of concern were measured using WBC count, INF-γ ELISpot, and Luminex multiplex cytokine analysis, and targeted gene expression profiling (NanoString immunopanel, qPCR). Another model, domestic cat WBVR (P31), was used for receptivity and transmission risk assessments, WBC counts, flowcytometry (CD4/CD8 phenotyping), and IFNy ELISpot from peripheral blood mononuclear cells (PBMCs).

Apart from measuring immunological parameters, certain studies in animal models from the partners also established the monitoring of physiological parameters during the challenge study, such as body core temperature, locomotion activity, and heart rate by implanting data loggers, as well as the determination of the daily water and energy expenditure. These data considerably improved our understanding of the disease progression, especially in animals that did not display any obvious clinical signs. This approach will therefore be continued in future studies.

As mentioned in section 2.1, the *in vitro* models for SARS-COV2 infection were performed by screening susceptible cell lines (Report, WP2-T2.2-ST2.2.1). In addition, the host-pathogen interaction parameters were mainly studied by microscopy (CPE) and whole transcriptome analysis (RNAseq) of viral and host.

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