Scientific report of short-term mission at Wageningen BioVeterinary Research (WBVR) at Lelystad, Netherlands

Ludivine Percevault

Genetics Viral and Biosecurity Unit

Ploufragan-Plouzané-Niort Laboratory, ANSES, FRANCE

Thesis supervisor: Béatrice Grasland, Ph.D

Thesis co-Supervisor: Maud Contrant, Ph.D

Head of Unit: Yannick Blanchard, Ph.D

Scientific and technical supervision in the WBVR:

- Rik de Swart, Ph.D
- Nora Gerhards, Ph.D
- Romy Dresken

My PhD thesis, which I realize in the Genetics Viral and Biosecurity Unit (UGVB) at the Ploufragan-Plouzané-Niort laboratory in Anses, in France, is focused on the porcine intestinal organoids as surrogate model for *In Vivo* experimentation on enteric swine viruses. The organoids are cells that self-organize in three dimensions and reflect the cellular, structural and functional diversity found within an organ. The purpose of my thesis is to analyse the contribution of the organoids on the study of host-virus interaction. For that, we have selected the porcine transmissible gastroenteritis virus (TGEV), an alphacoronavirus, as pathogen model. This virus causes anorexia, weight loss, diarrhoea and death in 100% of piglets less than two weeks old [1]. The main target organ of the virus is the enteric system[2]. In the UGVB, we have set up a functional infection protocol with the TGEV on porcine intestinal organoids. We have analysed the infection with two strains, the first one is a long term adapted cell culture strain (115 passages) in Swine Testicular cell lines. The second strain is contrarily poorly adapted to cell culture. We observed differences in infection capacity between of these two strains in intestinal organoids with a 3-log difference in virus produced was calculated in favour of the adapted strain. We are currently finalizing the analysis of host-virus interactions for both viral strains in intestinal organoids.

The short-term mission took place from June 30 to July 5, 2024 at the Wageningen BioVeterinary Research-Lelystad in Netherlands in Rik de Swart's team that has developed nasal and bronchial organoids expertise for different livestock's species including pig.
Some strains of TGEV can infect the respiratory system[3], the mobility in Rik de Swart's team then gave us the opportunity to develop additional knowledge in organoids and also the opportunity to test for the capacity of our strains to infect organoids from the swine respiratory complex for further comparison with our observations done in swine intestinal organoids.

During this week, I learned all the steps of the protocol, from the isolation of bronchial epithelial cells to the production of 3D organoids and the infection of cells.
For the first step of the protocol, I was able to practice preparation of bronchial epithelial cells from pig lungs. This protocol lasts 2 days, the first day consists in the recovery of the bronchial tubes from the lungs them in their dissociation in culture medium for epithelial cells isolation. The following day, epithelial cells are harvested and counted for subsequent freezing or culture. This protocol is very

different from the one we use for intestinal organoids and practising this protocol in a routine lab with expert was a real boost for my thesis program.
During the short term mission, I was also able to observe the production of 3D organoids. The aim of the experiment is to grow the cells in an extracellular matrix with a growth-factor-rich medium. With these culture conditions, the cells can proliferate and differentiate to reflect the cellular, structural and functional diversity found within the bronchial tubes. The rational is close to the production of intestinal organoids but it have significant different steps that are crucial for airway organoids production.
To infect the organoids, we need to make the viral receptor accessible for the virus. In fact, in 3D organoids, the TGEV receptor is at the apical pole, localized inside the organoid and therefore not accessible for the virus. To counteract this, one simple way to proceed is to transform 3D organoid in a cell monolayer. To reflect what is going on with bronchial tubes, an Air Liquid Interface (ALI) is realized to have the apical pole of the bronchial cells in contact with air. The cells are then grown in transwell plates fitted with a filter. I was able to learn how to use these plates and grow the cells. This air liquid interface cell culture system was a complete novelty for me, we spread intestinal organoids on classical plates with no air liquid interface.

The last step was to infect the cells grown on ALI with both TGEV strains of concern for my studies. I was able to carry out kinetics of infection: 0h, 24h and 72h.To optimize our infection conditions, I tested the impact of trypsin adjunction at the time of viral inoculation.
Even if the monolayer was unharmed, we observed floating cell debris only after 48h of infection. This debris were observed , with and without use of trypsin, in wells infected with the strain most suitable for culture. With the poorly adapted strain we observed cell debris only in wells inoculated with trypsin condition. Debris mean that some cells have died, suggesting a cytopathogenic effect linked to the infection but the cell monolayer has recovered allowing to extend the infection up to 72 hours. RNA extractions from cell washes, to see viral production, and from cells are underway to analyse whether TGEV strains have been able to productively infect bronchial organoids.

Thanks to the samples of infected bronchial organoids acquired during the short term mission, we will be able to compare ALI infections to those obtained with intestinal organoids by comparing the infection capacity of the two strains for the two tropisms and also the host-virus interactions obtained in the enteric and respiratory models if this last one is also productive.
This short-term mission was very rich and gave me the opportunity to learn all the steps of the protocol for producing and infecting bronchial organoids. I will now be able to transfer and teach the UGVB team in this technique. Mastering organoids for intestinal and respiratory tissues that represent the target organs for pathogens responsible of the main infectious pathologies of the pig industry, will provides us promising models to study and unravel the mechanisms involved in viral infections, in an attempt to understand and fight emerging, veterinary and economically important viruses.

References

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