VETBIONET

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

Deliverable D9.7

Development and validation of molecular tags to study the interaction of class 3 viruses including organ and cell type tropism and host responses

Due date of deliverable: M66

Actual submission date: M72

Start date of the project: March 1st, 2017 **Duration:** 72 months

Organisation name of lead contractor: Your organisation

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Dissemination level	
Public	**X**
Confidential, only for members of the consortium (including Commission Services)	
Classified, as referred to in Commission Decision 2001/844/EC	

Table of contents

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

1. Summary

Objectives:

The main objective of the task for which this deliverable was formulated, was to develop *in vitro* bioimaging approaches to assess host/pathogen interactions. The specific approach which was to be taken in this deliverable was to develop and validate the use of molecular tags in cattle, pigs and chickens, which would then be applied to study the interaction of class 3 viruses with organs/cells and host responses.

Rationale:

The activities reported in the present deliverable D9.7 are related to WP9 Task 9.4 and progress was followed through periodic updates as part of the consortium meeting schedules and at each periodic meeting. The two partners involved in this task, TPI (The Pirbright Institute) and AU (Aarhus University), sought to harmonize the ongoing activities in cattle and chickens (discussions during the 2nd annual meeting in Madrid, May 2019 and subsequently) and decided to focus on activated T and B cells, in line with the BCR and TCR sequencing activities carried out in WP8.2.

Results:

Successful development of phenotypic monoclonal antibody panels, as well as MHC class I tetramers were achieved and applied to chickens, cattle and pigs. However, combined application of the BCR and TCR sequencing methodologies developed in WP8.2 with the panels developed in this deliverable were not possible.

The main method of delivery of the key milestones was through publications, which are detailed below. The designed and validated staining panels have been as OMIPs (Optimized Multicolor Immunofluorescence) in Cytometry A and applications have been published as scientific papers:

- Cattle B-cell panel (OMIP-085): DOI: 10.1002/cyto.a.24683.
- Cattle T-cell panel (OMIP-089): DOI: 10.1002/cyto.a.24718
- Chicken T cell panel: DOI: 10.1002/cyto.a.24304
- MHC class I tetramers to identify porcine antigen specific CD8 T cells: DOI: 10.3389/fimmu.2023.1181716

Teams involved:

TPI (cattle and pig OMIP development / molecular tag application)

AU (Chicken molecular tag application).

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2. Introduction

T and B cells are known to be the major adaptive cellular effector arms of the immune response of vertebrate hosts to pathogen infection. Detailed examination of the B and T cell responses to infection and/or vaccination is essential in order to understand host-pathogen interactions which can be used to develop, amongst other elements, effective countermeasures, in particular vaccine development.

3. Results

3.1 Result 1

The cattle and pig activities were split into 5 sub-results:

1) Development of cattle B-cell OMIP: Monoclonal antibodies, which were freely available either within TPI or commercially available externally, were screened for reactivity in cattle PMBCs. Based on expression levels, co-localization profiles, and labelling intensity, these antibodies were directly conjugated with a selection of fluorochromes and multiplexed into a panel of antibodies. This panel was used to develop a standard approach of B-cell labelling in cattle which was published as an OMIP in cytometry A [1]. This was the first time a livestock OMIP has ever been published, and only the third species after human and murine.

2) Development and validation of cattle T-cell OMIP: Using the same approach as for the B-cells, monoclonal antibodies were identified, conjugated, assessed using cattle PBMCs and the OMIP published in cytometry A [2]. Subsequent to this assessment and publication, a polymorphism of CD4 (previously described in Italian herd within the breeds Italian Fresian, Pezzata Rossa Friulana, and in a Japanese herd of the breed Japanese Black) was detected in a British Holstein Fresian steer from a UK herd. Subsequent sequencing of the CD4 coding region of the host DNA confirmed this polymorphism, and a short communication is being prepared to describe this.

3) Assessment of OMIP antibodies to frozen tissue sections: Towards the end of the project, the mAbs in each published OMIP were screened individually using clean cattle pre-scapular lymph nodes (negative control for infection), which

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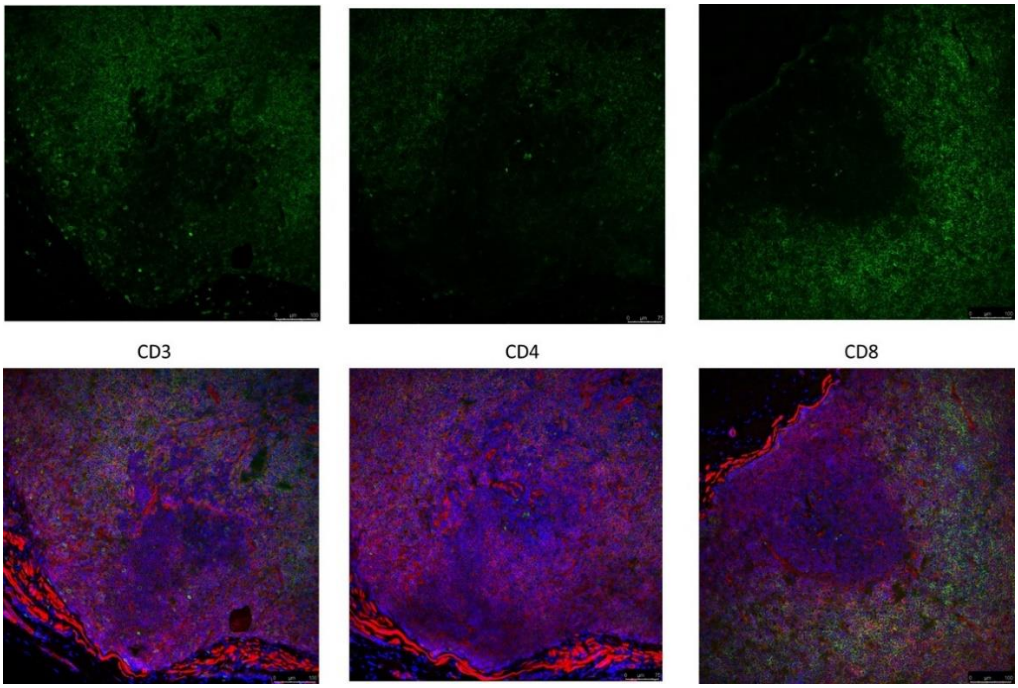
were embedded in OCT and cut on a cryo-microtome. Sections were then labelled with each of the mAbs (followed by an AF488 secondary polyclonal antibody) used in the OMIP with a view to multiplexing on tissues. Non-specific labelling of the secondary antibodies was undertaken by including a no first antibody control in each labeling experiment.

Results were that despite some autofluorescence in all lymph nodes tested, probably from macrophage inclusions, the secondary antibodies did not appear to cross-react with the tissues. An initial screen of each monoclonal was performed with the following reactivity noted:

- CD3 (MM1A), CD8 (CC63), CD21 (CC21 and CC51), MHCII (CC158), IgL (IL-A58), γδ TCR (GB21A), WC1 (CC15), CD4 (CC30 and CC8), CD25 (IL-A111), CD40 (IL-A158), and CD62L (IL-A58) all showed characteristic labelling patterns in the lymph nodes and were therefore considered to be successful and relevant.
- CD14 (CCG33), and CD71 (IL-A165) did not appear to have any reactivity in sections and were not considered useful for labelling cryosections.

Representative labeling patterns for 3 of the antibodies mentioned above (CD3, CD4 and CD8) are shown below – CD molecules in green, actin in red and nuclei in blue in the composite lower images. .



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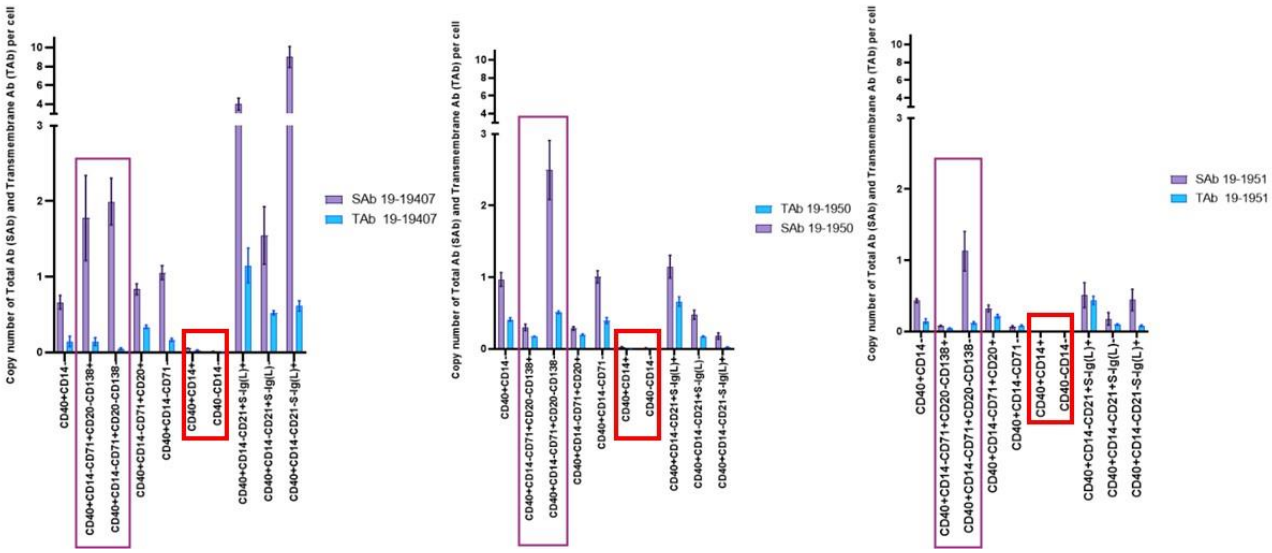
4) Application of OMIP panels to cattle PMBCs: B-cell subsets, as defined by the B-cell OMIP panel, was used to sort B-cells into multiple different categories based on their surface phenotype. A q-RT-PCR assay was developed which allowed the total antibody (SAb) and transmembrane (Tab) to be elucidated for each of these subsets. This helped to further define the CD40+ CD14 negative populations as B-cells, and rule out CD40+CD14+ and CD40-CD14- PBMCs as B-cells (Red box in the figure below). Moreover, there is evidence that CD40+ CD14- CD71+ CD20- CD138- cells may be plasma cells in cattle (purple box in figure below), in contrast to CD138+ cells in humans and mice. However, further functional assays are required to confirm this.



5) Application of MHCI tetramers to porcine PBMCs - as part of a TNA project (see D 15.1: "Harnessing local immunity for protection against influenza" - TNA user: M. Tenbusch): This TNA study examined the heterotypic response to influenza in pigs and whether it could be potentiated by IL1-B as previously observed in mice. MHC class I tetramers were created to bind swine influenza peptides as part of this task (T9.4), in order to enumerate swine influenza specific CD8 T-cells. A paper has been published (DOI: 10.3389/fimmu.2023.1181716)

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3.2 Result 2

Chicken activities:

Software-based panel design was performed at AU and initial validation was performed on the 11-color chicken T cell panel using commercial antibodies. Work on the chicken B cell panel was also undertaken, but the fact that there are very few commercial chicken B cell antibodies available limited our ability to develop a OMIP specific for B cells.

A solution of making a flexible panel that can be adjusted as needed to study the activation responses of chicken T or B cells was chosen, and a multiparametric protocol with simultaneous detection of 11 colors was developed. The idea of a separate panel for B cells was also abandoned, as only panels using commercially available reagents can be published in Cytometry A. Furthermore, an additional protocol was developed based on a monoclonal antibody against chicken CD154. This antibody was shared from TPI and incorporated in an assay to detect IFN-γ and IL-17 production in activated T cells by flow cytometry.

4. Conclusions

Cattle and Pig molecular tags (OMIPs and MHC Class I tetramers) : In this project, a successful program of work was conducted, which first optimized and published antibody panels to define cattle B and T cells in flow cytometry. This was the first time any farmed animal species has been featured in Cytometry A as an OMIP (DOI: 10.1002/cyto.a.24683; DOI: 10.1002/cyto.a.24718). These panels were then applied to determine usefulness and reactivity in cryosections of cattle clean lymph nodes on an individual basis, and also applied to an animal study to generate initial data on PBMC phenotype characterization during the course of FMDV vaccination. Moreover, the B cell OMIP panel was used to develop a q-RT-PCR assay for assessing B-cell antibody secretion status.

Pig antigen-specific (swine influenza) CD8 T-cells were identified by creation of MHCI tetramers as part of this work package (WP9) and applied to a TNA study conducted at TPI. A paper has been published (DOI: 10.3389/fimmu.2023.1181716)

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Chicken OMIPs: All optimization of the chicken flow cytometry panels resulted in a combined chicken T and B OMIP panel. A solution consisting of a flexible panel that can be adjusted to examine chicken T or B cell activation responses was chosen and a multiparametric protocol with simultaneous detection of 11 colors was developed. The OMIP was tested in a coronavirus experiment. One paper has been published [3](DOI: 10.1002/cyto.a.24304), two are in preparation.

5. Annexes

1. Roos, E.O., et al., *OMIP-085: Cattle B-cell phenotyping by an 8-color panel.* Cytometry A, 2023. DOI: 10.1002/cyto.a.24683.
2. Roos, E.O., et al., *OMIP-089: Cattle T-cell phenotyping by an 8-color panel.* Cytometry A, 2023. DOI: 10.1002/cyto.a.24718
3. Naghizadeh, M., et al., *Kinetics of activation marker expression after in vitro polyclonal stimulation of chicken peripheral T cells.* Cytometry A, 2022. DOI: 10.1002/cyto.a.24304
4. Schmidt, A. et al., *Effect of mucosal adjuvant IL-1β on heterotypic immunity in a pig influenza model.* Front Immunol., 2023. DOI: 10.3389/fimmu.2023.1181716

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