VVD-058

CLINICAL RESEARCH TOOLS FOR PRRSV GROWTH AND DETECTION

K. Heenemann [1], N. Schütze [2], A. Seydel [3], A. Rückner [1], M. Sieg [1], G. Alber [2], T.W. Vahlenkamp [1].

[1] University of Leipzig, Leipzig (Veterinary Faculty, Institute of Virology), Germany; [2] University of Leipzig, Leipzig (Veterinary Faculty, Institute of Immunology), Germany; [3] University of Leipzig, Leipzig (Veterinary Faculty, Institute of Bacteriology and Mycology), Germany.

Introduction

The porcine reproductive and respiratory syndrome (PRRS) is an economically important disease worldwide causing reproductive failure in sows and respiratory symptoms in piglets. The main target cells of PRRSV are alveolar macrophages (AMph). The growth of PRRSV *in vitro* is not consistently achieved in cell lines like MARC-145 cells. Primary porcine macrophages are susceptible to PRRSV. The aim of this project is to phenotypically characterize primary porcine macrophage subsets by flow cytometry and to investigate the individual cell subsets for PRRSV susceptibility.

Material & Methods

Monocyte derived macrophages (MoMph) were derived from PBMC after stimulation with M-CSF. AMph were obtained from pigs by bronchoalveolar lavage. MoMph, AMph and bone marrow derived macrophages (BMMph) were analysed by flow cytometry for their expression of CD172a, CD163 and CD14. PRRSV infection was verified by RT-PCR and immunofluorescence assay (IFA) using the monoclonal antibody SDOW17. MARC-145 cells were used for further passaging of PRRSV field strains after isolation in AMph.

Results

Flow cytometric analysis revealed different phenotypic expression of CD172a and CD163. Almost 100 % of the AMph were CD172a$^+$, in contrast to BMMph and MoMph with 50-66% and 48% CD172a+ cells, respectively. CD163 expression was observed on almost 100 % of the AMph as well as MoMph. In BMMph, CD163 expression was detected on 40-60% of the cells. CD14, a monocyte differentiation marker, was absent on AMph whereas almost all MoMph were CD14$^+$. About 20% of the BMMph were CD14$^+$.

Discussion & Conclusion

The phenotypic analysis of BMMph and MoMph revealed that only a minor fraction of the cell populations co-express CD172a, a marker for myeloid cells, and the PRRSV receptor CD163. Therefore further infection experiments focussed on CD172a$^+$CD163$^+$ AMph. PRRSV field strains showed different replication pattern. Further investigations intend to establish a robust *in-vitro* macrophage infection model for PRRSV field strains.