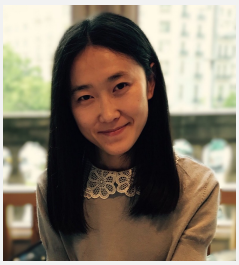
ImmunoTools *special* Award 2018



Yanli Li, PhD
PostDoc

Department of Animal Health and Anatomy, Faculty of Veterinary Medicine, UAB, V0-251, Barcelona, Spain

The role of exosomes in transmission of *Porcine reproductive and respiratory syndrome virus* (PRRSV)

Porcine reproductive and respiratory syndrome (PRRS) is one of the costliest diseases of pigs. The causative agent is PRRS virus (PRRSV1 and 2), an enveloped, positive-sense ssRNA virus. *In vivo*, PRRSV has a tropism for cells of the monocyte/macrophage lineage; and *in vitro*, certain types of dendritic cells have also been proved to be susceptible. During the course of infection, CD163 is thought to be the essential receptor which interacts with the glycoprotein (GP) trimers GP2-GP3-GP4 on viral envelope, resulting in the release of genome and the initiation of replication. However, in our study using bone marrow-derived dendritic cells (BMDC), the replication in apparently CD163-negative ($CD163^{-}$) cells were observed, by flow cytometry and confocal microscopy analysis. The further sorting experiment based on the expression level of CD163 showed that $CD163^{-}$ cells were infected only when $CD163^{low}$ or $CD163^{high}$ cells were included in the culture. Thus, in our opinion, the susceptible $CD163^{-}$ might arise as a result of milieu created by the infected $CD163^{low/high}$ BMDC. Exosomes carrying the viral genome are the hypothesized candidate.

Exosomes are a distinct population of microvesicles ranging from ~30 to 150 nm in size. Recent evidence indicates there is a role for exosomes in intercellular communication through transfer of proteins, mRNAs and microRNAs. However, due to the technical limitation, their role in the transmission of infectious pathogens is less known, and much less known for pathogens causing pig diseases.

Based on the previous data, the project I am being engaged aims to study the role of exosomes in the transmission of PRRSV. For that, three main problems need to be solved. First, rpGM-CSF-BMDC cultures have been widely used as DC *in vitro* to study the host-pathogen interaction. They were also employed in our previous studies. But these DC do not represent *bona fide* DC, but rather the inflammatory DC. Thus in our following work, the *bona fide* DC will be developed, for which rhFlt3L/CD135 will be needed, and for comparison rpGM-CSF will be included. The generated DC then will be phenotyped and classified based on the expression of CD1a, CD4, wCD11R1 (CD11b), CD14, CD16, CD123 (IL-3R), CD135 (Flt3), CD163, CD172a and CADM1. For the purpose of stimulation or inhibition of some functions of DC, TNF-alpha, rpIL-2 and rpIL-10 will be used. Second, exosomes will be isolated from the cultures of infected or uninfected porcine alveolar macrophages (PAM) or Flt3L-DC. After

isolation, the quality of exosomes will be assessed including: 1) the size, by fluorescence Nanoparticle Tracking Analysis and cryo-electron microscopy; 2) the exosome-enriched markers by Western Blotting or some by flow cytometry/virometry. These markers include tetraspanins CD9, CD63 and CD81; the endosomal sorting complex required for transport proteins, TSG101, CHMP4A-B and VPS4B; endosome or membrane-binding proteins Annexins; and signal transduction or scaffolding protein syntenin; 3) the level of proteins not expected to be enriched in exosomes. These proteins include those associated with endoplasmic reticulum, for instance HSP90B1 and calnexin; with Glogi, for instance GM130; with mitochondria, for instance cytochreome C; with nucleus, for instance histones; and with Argonaute/RISC complex, for instance AGO). Thirdly, the exosomes will be used to infect the macrophages or *bona fide* DC based on the expression of receptors (CD163, Siglec-1 and Siglec-10) required for PRRSV infection, to see whether exosomes are able to transmit PRRSV infection.

Based on the work described above and the reagents available in the list, I intend to choose the reagents including rh Flt3L/CD135, rp GM-CSF; rp IL-2, rp IL-10, rp TNF-alpha; APC-conjugated anti-human CD14, and the corresponding isotype; FITC-conjugated anti-human CD63, and the corresponding isotype; APC-conjugated anti-human CD9, and the corresponding isotype; PE-conjugated anti-human Annexin V, and the non-conjugated anti-human Annexin V.

Although a pool of antibodies and proteins are needed for my study, because very few of the listed reagents are specific for pigs, thus what I can choose is very limited. If there are more reagents for pigs, it will be very helpful to my study.

ImmunoTools *special* AWARD for **Yanli Li** includes 12 reagents

FITC - conjugated anti-human CD63 and the corresponding isotype

PE - conjugated Annexin V and the non conjugated Annexin V

APC - conjugated anti-human CD9, CD14 and the corresponding isotypes

recombinant human cytokines: rh Flt3L/CD135

recombinant porcine cytokines: rp GM-CSF, rp IL-2, rp TNF-alpha

DETAILS more AWARDS