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An ultrastructural study of conventional 'cell to cell contact' *in vitro* swine influenza virus infection

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Influenza viruses are enveloped, single stranded RNA viruses in the family *Orthomyxoviridae*, and causes sub-acute or acute respiratory infection in swine farms. Pigs play a crucial role in the interspecies transmission of influenza viruses, they can act as "mixing vessels" for new influenza strains. In fact, swine population are susceptible to avian and human influenza strains, and have been recently demonstrated that these virus can easily cross the species barrier, as it has happened with the new A (H1N1) 2009 pandemic virus. In the other hand, the dendritic cells (DCs) mediate the induction of immunity to pathogens, but their interaction with SwIV is not fully characterized. The main aim of the present study was to evaluate the interaction between porcine DCs and SwIV and the possible role by the former in being carriers of porcine influenza virus using ultrastructural and immunolocalization evaluations by transmission electron microscopy (TEM).

Bone marrow hematopoietic cells were obtained from femurs of healthy Large white X Landrace pigs of eight weeks of age, free from porcine reproductive and respiratory syndrome virus (PRRSV) and negative to type-2 porcine circovirus (PCV2) and influenza virus. Bone marrow dendritic cells (poBMDCs) where generated in an eight days protocol as previously described by (Kekarainen et al., 2008), and previously to be infected, morphologically evaluated. Influenza virus infection has been thoroughly investigated on permissive epithelial cells as MDCK, in this sense SwIV poBMDCs infected and the interaction MDCK cells was analyzed. The poBMDCs cells were exposed *in vitro* to a conventional circulating strain of SwIV, H3N2. The DMEM infected was taken to incubate MDCK. Infected and control poBMDCs and MDCK cells were fixed and processed for conventional and immunogold labelling EM studies. The inmunomarker was made with the monoclonal antibody to influenza A virus.

The ultrastructural evaluation revealed abundant 80-100 nm virus like-particles resembling influenza virus inside vesicles but also freely in cytoplasm in infected poBMDCs. Remarkably, several SwIV like-particles freely in the cytoplasm were observed in close contact with Golgi complex, budding from internal cistern of the Golgi complex membrane to the trans-Golgi network. By contrast with SwIV-infected poBMDCs, in MDCK mature SwIV like-particles were observed in the extracellular space next and attached to cellular membrane. In the cytoplasm of MDCK, small and large vesicles were observed containing mostly immature SwIV like-particles (± 80 nm). Viral particles from infected-poBMDCs were able to induce cytopathic effect in susceptible cells only when cell-to-cell interaction was favored. Considering that this is the first report about the fine interaction of porcine influenza viruses with poBMDCs, these data may help understanding the role of DCs as important antigen presenting cells in the pathogenesis and epidemiology of influenza virus and the role of pigs as virus reservoirs with medical interest.

1. Heinen, 2002.
2. Horimoto and Kawaoka, 2001.
3. Lipatov et al., 2004.
4. Garten et al., 2009