Labelling of porcine circovirus and ultrastructural changes in histiocytes from naturally infected pigs: a tool for diagnosis?

C. Rodríguez-Cariño[1], A. Sánchez-Chardi[2], J. Segalés[1]

1. Departament de Sanitat i Anatomia Animals. Centre de Recerca en Sanitat Animal (CReSA), UAB-IRTA, Campus de la Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain.
2. Servei de Microscopia, Facultat de Ciències. Campus de la Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain.

carolina.carino@cresa.uab.cat
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The postweaning multisystemic wasting syndrome (PMWS) is a porcine disease characterized by lymphocyte depletion and granulomatous inflammation mainly in lymphoid tissue. Although this pathology caused by the porcine circovirus type 2 (PCV2) has important economic aspects, there is limited information regarding ultrastructural alterations due to PMWS, virus cycle and subcellular localization of virus particles in cells of naturally affected animals. Taking into account this lack of extensive ultrastructural studies on PMWS lymphoid tissues, the aims of the present work were to evaluate the ultrastructural lesions in lymph nodes of diseased animals and to correlate these alterations with the viral localization assessed by immunolabelling. For this purpose, small pieces of mediastinal and inguinal lymph nodes from healthy and PMWS affected pigs were processed for electron microscopy studies (conventional and immunogold labelling). Firstly, samples were fixed with 2% paraformaldehyde and 2.5% glutaraldehyde, postfixed with osmium tetraoxide, dehydrated with acetone and embedded in Epon resin for conventional methods. Duplicate samples were fixed with 4% paraformaldehyde and 0.1% glutaraldehyde, cryoprotected with sucrose, cryofixed with liquid propane, dehydrated and embedded in Lowicryl HM20. Ultrathin sections were labelled with PCV2 anti-cap as primary antibody, and IgG coupled with 10 nm colloidal-gold particles as secondary antibody. Important ultrastructural alterations were only noted in lymph nodes from PMWS affected pigs, mainly in histiocytes but also in lymphocytes, and endothelial and dendritic cells. In contrast, the cells of control animals showed normal shape and structure. The histiocytes showed severe swelling and proliferation of mitochondria, proliferation and dilation of rough endoplasmic reticulum (RER) and Golgi complex, and lyzosome proliferation in PMWS pigs. These infected histiocytes also contained large numbers of electron-dense intracytoplasmatic inclusions (ICIs) bodies with viral-like particles (VLPs) (**Figure 1.1**). They had round, oval or irregular shapes, with variable electron-density and they were usually dispersed throughout the cytoplasm; their diameter varied from 0.1 μm to 4.5 μm. Some cells had intranuclear inclusions (INIs). Large and small ICIs showed VLPs with 8 to 17 nm in diameter, in granular arrange or paracrystalline arrays with membrane fragments (**Figure 1.2**). ICIs were usually finding arrange about mitochondria (mit) (**Figure 1.3**). Severe immunolabelling were noted in ICIs with VLP to PCV2 anti-cap (**Figure 1.4**), as well as degenerated mitochondria which they were labelled in outer and inner membranes. Virions with different forms and sizes and subcellular changes were found in the cytoplasm of histiocytes. Our data suggest that membrane structures of organelles might be used for viral replication and viral factory formation. These findings are consistent with previous data obtained for other viruses, supporting our hypothesis of PCV2 cycle in histiocytes. Therefore, it seems that histiocytes

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play a significant role on PMWS pathogenesis. The results of immunogold labelling studies have corroborated all changes evaluated by conventional methods indicating the suitability of comparative analyses with these complementary techniques.

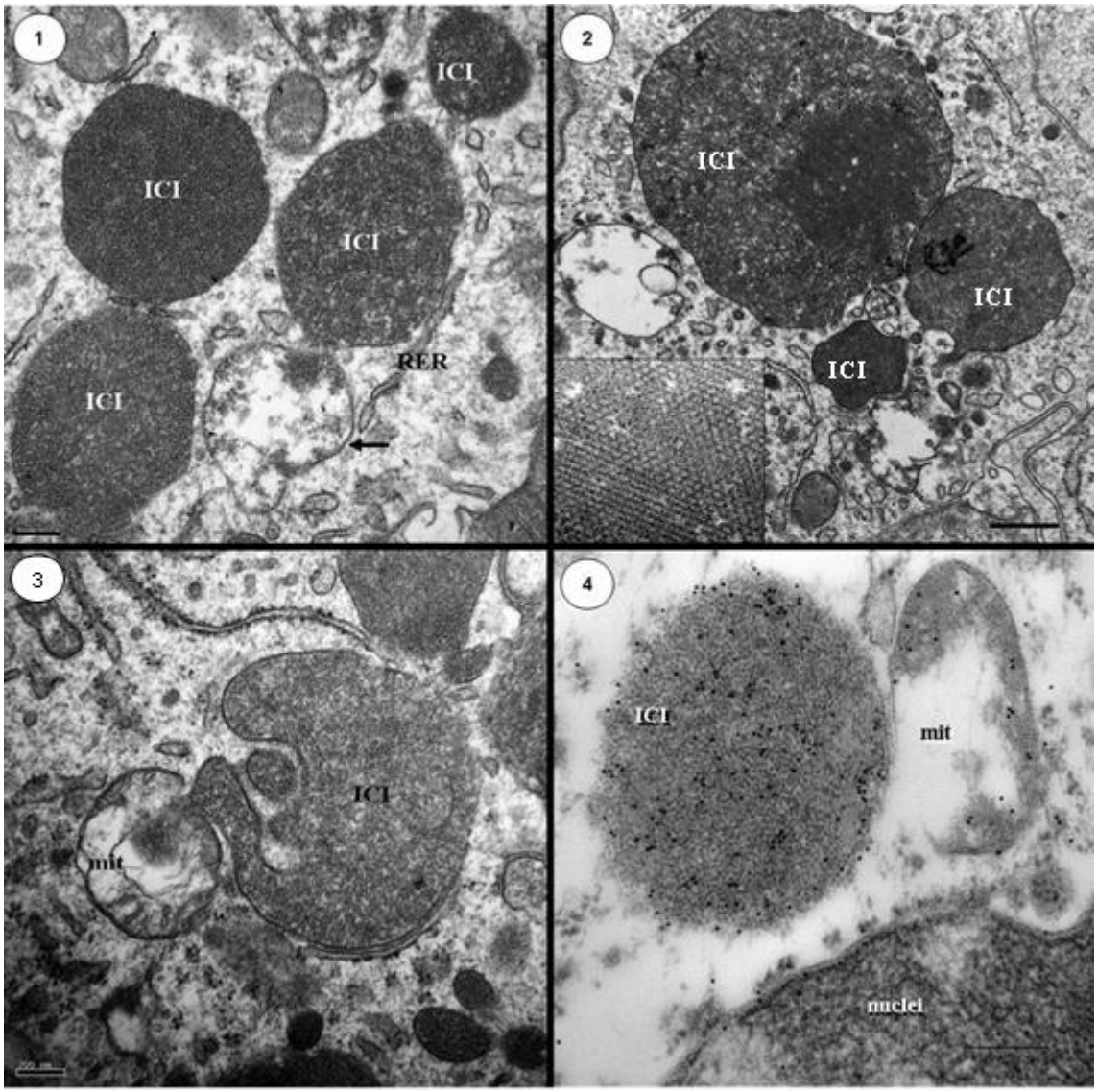

Figure 1. Representative electromicrographs of lymph nodes from infected animals showing ultrastructural changes in histiocytes and virus labelling

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