VIRAL DISEASES

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THE UPTAKE AND DISINTEGRATION OF PORCINE CIRCOVIRUS TYPE 2 VIRIONS IN BLOOD MONOCYTIC CELLS

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Monocytic cells are important target cells for porcine circovirus type 2 (PCV2) in pigs. They can take up PCV2. However, the mechanism of this process and the outcome were still not elucidated. To examine the mechanism of PCV2 uptake by monocytic cells, blood monocytes were isolated by plastic adherence, pre-treated with different endocytic inhibitors for 30 min at 37 °C and inoculated with PCV2 in the presence of the same inhibitors for 1 h to allow the uptake of virus particles. Then, PCV2 antigens and cell contours were visualized by a double immunofluorescence staining, and the level of uptake was quantified using Image J software. The uptake of PCV2 was significantly decreased by (i) chlorpromazine (84±7 % reduction), (ii) cytochalasin D (82±11 % reduction), and (iii) dynasore (50±24 % reduction), which indicated that the particles were internalized via clathrin-mediated, dynamin-dependent endocytosis. In contrast, inhibiting macropinocytosis with amiloride and caveolae-dependent endocytosis with methyl-β-cyclodextrin or filipin did not affect the PCV2 uptake. To examine the fate of the virions after uptake, cells were inoculated with PCV2 for different periods before they were fixed and stained. Afterwards, PCV2 antigens were quantified using Image J software. After the uptake, a disassembly of the virions was observed up till 12 hours post inoculation, after which a low level of antigens remained present. In the future, it will be examined if PCV2 genomes become transported to the nucleus and if they become transcribed.