利用特定抗體偵測豬第二型環狀病毒的殼鞘蛋白質及開放閱讀框架三蛋白質

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摘要

豬第二型環狀病毒(PCV2)為最小且含單股環狀去氧核醣核酸基因組的無封套病毒，該病毒常造成造成豬隻離乳後多發性消耗症候群，而淋巴球減少是 PCV2 感染的特徵之一。PCV 含有三個主要開放閱讀框架(ORF)：ORF1 能轉譯病毒複製相關蛋白質；ORF2 能轉譯具有引起免疫性的病毒殼鞘蛋白質；ORF3 能轉譯具有引起細胞凋零的蛋白質。PCV2 的 ORF3 蛋白質能與 Pirh2 (使 p53 普存化的 E3 黏合酶)相互作用，導致 Pirh2 數量減少並增加 p53 數量，促成病毒感染細胞的凋零化。本試驗的目的是偵測 PCV2 相關蛋白質來了解病毒與淋巴球的交互關係。4 頭 14 週齡的 SPF(排除 PCV2 清淨)豬飼養在生物安全性等級 3 之動物舍。每 2 或 3 週採血一次，並利用 Ficoll-Paque 密度離心方式分離週邊血液單核球(PBMC)。將 PBMC 做成螢光染色分析(FA)的玻片或細胞溶解液。利用不同單株或多株抗體組成抗原捕獲酵素連結免疫吸附法(AC-ELISA)來檢測細胞溶解液中 PCV2 殼鞘蛋白質。在間接螢光染色分析中，利用不同單株抗體(抗殼鞘蛋白質或開放閱讀框架三(ORF3)蛋白質)都可以在豬淋巴球發現陽性訊號。利用單株抗體抗 ORF3 蛋白質及多株抗體抗殼鞘蛋白質觀察 PCV2 感染細胞內病毒相關蛋白質的位置分布，這些具有雙重陽性染色反應的細胞，其細胞核呈現較小、不規則或碎裂狀。

The capsid protein and ORF3 protein of porcine circovirus type2 were detected by specific antibodies

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Abstract

Porcine circovirus 2 (PCV2) is a small, non-enveloped virus with a single-strand circular DNA genome. It is the causative agent of postweaning multisystemic wasting syndrome in pigs. Lymphocyte depletion is a hallmark of PCV2 infection. The PCV2 genome encodes for three major open reading frames (ORF), which code for the replication associated proteins (ORF1), immunogenic capsid protein (ORF2), and an apoptosis inducing protein (ORF3). The ORF3 protein of PCV2 interacts with Pirh2, an E3 ligase involved in the ubiquitination of p53, resulting in the decreased levels of Pirh2 and an increase in levels of p53, and leading to apoptosis of the virus-infected cells. The aim of this study was to provide a new profile about the lymphocyte interaction with PCV2 by detecting PCV2 associated proteins. Four 14-week old SPF (excluding PCV2) pigs were kept in one isolated room of the institute BSL3 containment facility. Blood was collected every two or three weeks. Peripheral blood mononuclear cells (PBMC) were isolated from the buffy coat fraction of blood by density centrifugation over Ficoll-Paque. PBMCs were prepared for making fluorescence assay (FA) slides and cell lysate. PCV2 capsid proteins of cell lysate were detected with the antigen-capture enzyme-linked immunosorbent assay (AC-ELISA) by using different monoclonal antibodies (MAbs) and polyclonal antibodies (PAb). In indirect FA, MAbs (anti capsid protein or ORF3 protein) showed positive signals on those swine lymphocytes. Positive FA signals revealed by both of the MAb anti ORF3 protein and PAb anti capsid were shown on the same cellular compartments. The dual positive signals staining cells displayed small, irregular or fragmented nuclei.