

抗豬第二型環狀病毒殼鞘蛋白質單株抗體之研發

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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糖核酸基因組的無封套病毒，該病毒常造成養豬產業的重大經濟損失。目前，已確認 PCV2 有兩種主要的基因型：PCV2a 和 PCV2b。通常患有豬第二型環狀病毒相關疾病（PCVD）的豬隻較常分離出 PCV2b，據文獻指出 PCV2b 比 PCV2a 更具毒力。

本研究的目的是生產特殊抗體，其具有抗 PCV2b 開放閱讀框二（ORF2）蛋白質（又稱殼鞘蛋白質）的能力，並利用這些抗體開發成該疾病的診斷工具，能偵測 PCV2b 抗原。將 BALB/c 小鼠免疫 PCV2b 殼鞘胜肽數次。隨後將小鼠安樂死，取其脾臟細胞與骨髓瘤細胞融合。取融合瘤上清液進行酵素聯結免疫吸附法（ELISA）檢測，篩選抗 PCV2b 特異性抗體。融合瘤經反覆的限制稀釋及 ELSIA 檢測篩選，進而篩選穩定的融合瘤細胞且能分泌抗 PCV2b 單株抗體，這些單株抗體並進行特性分析，其中有八株單株抗體為免疫球蛋白 M，六株單株抗體為免疫球蛋白 G₁。以商品化的 PCV-2 螢光染色用玻片（VMRD®）檢測這些單株抗體，其中有五株單株抗體呈陽性信號。

Generation of monoclonal antibodies recognizing the capsid protein of porcine circovirus type 2b

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Abstract

Porcine circovirus 2 (PCV2) is a small, non-enveloped virus with a single-strand circular DNA genome that has had severe impact on the swine industry. Currently, two main genotypes of PCV2 have been recognized: PCV2a and PCV2b. PCV2b has been more frequently isolated from pigs with PCV-associated diseases (PCVAD) and may be a more virulent subtype than PCV2a.

The purpose of this study was to generate antibodies anti ORF2 protein (capsid protein) of PCV2b for developing diagnostic tools which can detect PCV2b antigens. BALB/c mice were immunized with capsid peptides of PCV2b. Mice were subsequently euthanized and spleen cells were fused with myeloma cells. Hybridoma supernatants were removed and screened for the presence of PCV2b-specific antibodies by an enzyme-linked immunosorbent assay (ELISA). After repeatedly cloning by limiting dilution and screening by ELISA, stable hybridomas secreting anti-PCV2b monoclonal antibodies (MAbs) were obtained and characterized. Eight clones secreted the IgM class of antibody, whereas six clones have the IgG₁ isotype. Five MAbs produced positive signals with PCV-2 FA Substrate Slide (VMRD®).