

## Apoptosis of lymphocytes in piglets after porcine circovirus 2 inoculation *in vitro*

**Abstracts:[Objectives]** Porcine circovirus 2 (PCV2) can induce lymphocytes depletion in the immune organs, which is the basis of piglets immunosuppression. This paper studied the interactions between PCV2 and piglets lymphocytes *in vitro*, and tested the changes of lymphocytes proliferation, apoptotic rate as well as the expression of Caspase-3 at different culture times. These results contribute to reveal the main regulative mechanism of lymphocyte apoptosis after PCV2 infection. **[Methods]** Four conventional piglets with free of PCV2 antibody were used in this study. Lymphocytes were isolated from spleen in the sterile operation. Single cell suspension was cultured at 24-well plates,  $5 \times 10^6$  cells/well. The cells were randomly divided into experimental group and control group. In experimental groups, 200  $\mu$ L virus suspension of PCV2 was added in each well, while the equivalent cell-culture medium was added in control group, Cells were cultured at 37 °C and were collected at 0, 2, 4, 6, 12, 24, 36, 48 h, respectively. After washing with PBS, the cells were saved. Cell apoptosis were detected by agarose gel electrophoresis and observed by electronic microscope. The changes of cell cycle, apoptotic rate and the expression of Caspase-3 were measured by flow cytometry (FCM). **[Results]** 2–48 h after PCV2 inoculation, the specific DNA ladder of apoptosis was detected by agarose gel electrophoresis. In control group, there was no ladder appeared at 0 and 6 h. Lymphocytes showed no ultrastructural changes at 6 h in control group. The early changes of apoptosis started to appear in manipulus cells until 8 h. In experimental group, early changes of apoptosis, such as microvilli disappearing, nuclear enrichment, chromatin condensation and condensation around the membrane, were observed as early as 2 h in lymphocytes, followed by cytoplasm condensation, cell volume reduction, endoplasmic reticulum dilatation, forming a series of expansion vesicles, cytoplasmic organelles aggregation, cell membrane gradually invaginated, the cells were divided into a plurality of discontinuous apoptotic bodies in variable sizes. The FCM results showed that apoptotic rate of lymphocytes was increased along with the culture time in both experimental and control group; after 4 h, apoptotic rate of lymphocytes in experimental group were significantly higher than that in control group ( $P < 0.05$ ); time-dependent decrease of proliferation index (PI) was detected in both two groups with significantly lower in experimental group than that in control group at 2, 4, 6, 12, and 24 h ( $P < 0.05$ ). The results of FCM detection showed that the number of lymphocytes, which express Caspase-3, was increased in along with the culture time in both control and experimental group. The percentages in experimental group were significantly higher than that in control group during the whole detection time ( $P < 0.05$ ). After cultured for 6 h and 48 h, the percentage of lymphocytes, which express Caspase-3 was 7.75% and 17.42% respectively in experimental group, in comparison with 3.60% and 5.33% in control group. **[Conclusion]** PCV2 infection could induce lymphocytes apoptosis and inhibit proliferation of piglet's lymphocytes *in vitro*. Caspase-3 is an important regulator in PCV2-induced lymphocytes apoptosis.

**Keywords:** Caspase-3; apoptosis; porcine circovirus 2 (PCV2); lymphocyte; piglet