Th17 Cell Differentiation Induced by Cytopathogenic Biotype BVDV-2 in Bovine PBLCs

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Abstract

Background: Bovine viral diarrhea virus (BVDV) is a major pathogen that causes bovine viral diarrhea/mucosal disease (BVD-MD), which has become a global infectious disease due to its wide spread and the lack of effective treatment. The process of BVDV infection is complex. Once infected, host immune cells are activated and modulated. As a major immune cell, peripheral blood lymphocyte cells (PBLs) are the primary target of BVDV. In order to further understand the mechanism of BVDV- host interaction, the expression profiles of host lymphocytes mRNAs associated with BVDV infection were investigated by transcriptomic sequencing analysis.

Results: The transcriptomic sequencing analysis was performed on bovine PBLs infected with CP BVDV-2 GS2018 after 12 h of infection. Gene expression profiling demonstrated that 1,052 genes were differentially expressed in GS2018 infected PBLs compared with the control group. Of these genes, 485 genes were up-regulated and 567 were down-regulated. 19 differential expressed genes (DEGs) were selected for validation using quantitative real-time PCR and the results were consistent with the results of RNA-Seq. Gene ontology enrichment and KEGG pathway analysis showed that 1052 DEGs were significantly enriched in 16 pathways, including cytokine-cytokine receptor interaction, IL17 signaling pathway, PI3K-Akt signaling pathway, MAPK signaling pathway and TNF signaling pathway. PPI network analysis showed that IL17A, IFN- γ and TNF- α interacted with various proteins and may play crucial roles in BVDV-2 infection. Of note, we confirmed that GS2018 induced Th17 cell differentiation in PBLs and persistently increased the expression levels of IL17A. In turn, the replication of GS2018 was inhibited by IL17A.

Conclusion: In this study, the transcription changes of DEGs related to host immune responses in bovine PBLs were caused by CP BVDV-2 infection. In particular, the effector molecules IL17A of Th17 cells were significantly up-regulated, which inhibited viral replication. These results will contribute to exploration and further understanding of the host immune response mechanism and interaction between host and BVDV-2.

Full Text

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Tables

Due to technical limitations, tables are only available as a download in the Supplemental Files section.

Figures

Figure 1

Heat map and volcano plot of the DGEs between control and BVDV-2 infected group at 12 h. a. Heat map display the red represent the significantly up-regulated genes and the green represent the significantly down-regulated. b. volcano plot show the red represent the significantly up-regulated genes and significantly down-regulated genes in blue.

Figure 2

Verification of the mRNA level of some DEGs by qRT-PCR. a. The results of RNA-Seq. b. The results of qRT-PCR. GAPDH was used as endogenous internal reference gene and the relative content was calculated according to the $2^{-\Delta\Delta C_t}$ method.

Figure 3

GO annotation for the DEGs between control and BVDV-2 infected bovine PBLs. Horizontal axis represents the number of genes annotated on GO terms. Vertical axis represents the GO terms of BP, CC, and MF. Red represent the genes of up-regulated and the blue represent the down-regulated genes on different GO categories.

Significant Enriched Pathway Terms(top 20)

Figure 4

KEGG pathway enriched pathway analysis of DEGs with top 20 enrichment scores.

Figure 7

Th17 cells differentiation was detected by flow cytometry. a. Percentages of Th17 cells labeled by the CD4 and IL17A monoclonal antibodies. b. The bar chart shows the percentage of Th17 cells in control and GS2018 infection group at 6 h, 12 h and 24 h, respectively. Data are representative of three independent experiments and presented as means $\pm$ SDs. (ns, significant; * $p < 0.05$; ** $p < 0.01$).

Figure 8

The expression of IL17A was detected by qPCR and Western blot in PBLCs. a. qPCR detection at 12 h, 24 h, 48 h and 72 h, respectively. b. Western blot detection at 12 h, 24 h and 48 h after GS2018 infection,

respectively. The gray analysis of western blot result shows in bar chart. Data are representative of three independent experiments and presented as means $\pm$ SDs. (ns, significant; * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$; **** $p < 0.0001$).

Figure 9

Overexpression of IL17A inhibited the replication of GS2018. The pEGFP-N1-IL17A and pEGFP-N1 fluorescence signal was detected by laser confocal microscope at 24 h (a). The IL17A (b) and 5'UTR (c) were detected by qPCR. The BVDV-2 E2 (d) were detected by western blot. The gray analysis of western blot result shows in bar chart. Data are representative of three independent experiments and presented as means $\pm$ SDs. (ns, significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$).

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