The Effect of Bovine Viral Diarrhea Virus (BVDV) Persistent Infection on Gene Expression and Pro- and Anti-inflammatory Cytokine Levels

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Introduction

Bovine Viral Diarrhea Virus (BVDV) is a major viral affliction of the cattle industry in the United States. BVDV infections are responsible for the largest number of respiratory and reproductive viral disease cases of cattle in the US. BVDV can cause explosive outbreaks of severe acute disease that can result in high mortality and mucosal disease. BVDV can also cause persistent infection (PI) in animals resulting in individuals that shed virus life-long and cause long-term health and reproductive problems in the herd. Strains of BVDV that cause severe disease have a greater impact on infected macrophage production of cytokines and the capacity to injure adjacent tissue. The goal of this project was to identify the effect of PI on the immune gene expression and pro- and anti-inflammatory cytokine levels in persistently infected animals.

Materials and Methods

Serum samples were collected monthly for a three-month period, from six BVDV persistently infected (PI) calves and six age-matched healthy calves at the SDSU Veterinary Science Research Unit. MDM supernatant harvested on day 6 and sera were isolated from each animal and assayed using the ELISAs. MDM and sera were tested for levels of four cytokines: pro-apoptotic cytokines TNF-_, anti-inflammatory cytokine, IL-10, and the proliferative cytokine IFN-_, and IL-4. Separate ELISAs for each cytokine were utilized in this experiment. The TNF-_, IFN-_, and IL-4 were commercial ELISA kits (Endogen, San Diego) while the IL-10 assay was adapted from the method of Kwong et al (Vet Immunol Immunopathol. 2002;85:213–23). Bovine alveolar cells were obtained following bronchial lavage of either from six BVDV persistently infected (PI) calves and six age-matched healthy calves from the SDSU Beef Breeding Unit. Cells were harvested and RNA extracted and amplified (aRNA). The aRNA was hybridized to two different bovine microarrays obtained from the Center for Animal Genomics at Michigan State University. One array contained 8400 genes and the other 750 genes. Hybridization was measured by fluorescence and statistically analyzed.

Results

The IL-10 levels of the health calves remained slightly higher than the PI calves' IL-10 levels during the three-month period. The IL-4 levels in the sera were higher in the PI animals than the normal animals. However the IL-4 levels decreased in both groups over the three time points. The IFN-_ levels of the PI calves are significantly higher than the normal animals. The TNF-_ levels in the sera decreased over the three months and remained below the TNF-_ levels of the normal animals. For the microarray analysis, three groups of genes were different between the PI animals and the control animals: cell death, immune and cell organelles. Several immune genes were down regulated including interferon, pro-inflammatory and antigen presentation. The cell death-apoptosis pathways were also enhanced.

Significance

PI cattle frequently succumb very quickly and in our previous studies ~25% of PI animals that die had no lesions. PI cattle have several different cytokine pathways that were affected indicating a decrease in inflammatory response and likely a decrease in the overall immune response of PI cattle. This was further supported by the microarray experiments. The increase in cell death pathway genes expression may help explain why PI animals have such a short life span. This research indicates that eliminating the PI animal after diagnosis is likely the most prudent practice.