

Isolation and preliminary characterization of bovine Th17 lymphocytes

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ABSTRACT BOOK

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P022

Predicted microRNA biomarkers of *Mycobacterium avium* subspecies *paratuberculosis* using an *in-silico* bioinformatics approach

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Mycobacterium avium subspecies *paratuberculosis* (MAP) is the causative agent of Johne's Disease (JD) or paratuberculosis, a chronic granulomatous enteritis affecting ruminants. Typified by long and silent subclinical disease phases, there is a defined need for the early detection and diagnosis of MAP infection. Biomarkers are defined as objectively measurable indicators of normal or disease processes and may provide further insight into the interplay between host immune systems and pathogenic bacteria. microRNA (miRNA), a small non-coding subset of RNA, have been proposed as potential biomarker targets as they are highly stable and exert control over protein translation and gene expression. Previous studies have shown modulation of miRNA responses to mycobacterial infection, making them ideal candidates for biomarkers for the diagnosis and differentiation between disease states of paratuberculosis. This study aimed to generate a list of candidate biomarkers for the diagnosis of paratuberculosis in cattle and sheep using bioinformatic predictions and Ingenuity Pathway Analysis software (IPA).

A full literature search was carried out for the terms "Mycobacteria + paratuberculosis + miRNA + microRNA + mycobacterium" using the Scopus database and Abcam's Firefly Discovery Engine. A list of potential miRNAs was compiled and uploaded to IPA. A core analysis was run, and new miRNAs added based on suggested function and commonalities between networks. Key networks were generated based on function and relevance to MAP pathogenesis.

Eight key networks were obtained from the original core analysis and a list of candidate biomarkers for identifying MAP infection was generated.

This list of candidate biomarkers for MAP infection provides potential miRNA biomarkers that may aid in the diagnosis of paratuberculosis in sheep and cattle. These candidates will not only provide the basis for future investigations, but may also further elucidate the molecular mechanisms of MAP pathogenesis through their associated modulation of target molecules and immunological pathways.

P023

Isolation and preliminary characterization of bovine Th17 lymphocytes

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Interleukin 17A-producing T helper cells (Th17) are effector memory CD4+ T cells that are crucial to adaptive immunity to extracellular bacteria. The activities of these cells in the bovine species are not yet defined for want

of straightforward cultivation and isolation procedures. We have developed a method to cultivate, expand, sort and maintain in culture bovine Th17 cells from circulating CD4+ T cells of adult cows. Using polyclonal stimulation (antibodies to CD3 and CD28), we expanded positive CD4+ IL-17A+ T (Th17) cells in a cell culture medium without serum supplemented with TGF-β1 and IL-6. We used IL-2 to expand the cells, which were characterized by intracellular labeling for IL-17A and IFN-γ. Then, we isolated populations of CD4+ T cells producing IL-17A, IFN-γ or both by labeling surface IL-17A with either a complex of biotinylated anti-CD45 antibodies-streptavidin-biotinylated anti-IL-17A antibodies or by direct surface labeling with antibodies to IL-17A, followed by flow cytometry cell sorting. The percentages of surface-labeled IL-17A-secreting cells were quite similar to the percentages of intracellular-labeled IL-17A-producing cells of the same cultures, and the two labeling procedures of live cells yielded similar results. The sorted IL-17A+ cells were restimulated and expanded. After expansion, 80% of the isolated Th17 cells were positive for IL-17A intracellular labeling. The sorted IL-17A+ cells can be frozen, stored and expanded again. The sorted Th17 cells secreted much more IL-17A and IL-17F than did CD4+ IL-17- cells. Notably, most of Th17 cells secreted IFN-γ, although in lower amounts than did CD4+ IL-17- cells. Sorted cells were characterized by transcriptomic profiling. Genes coding for Th17 signature cytokines (IL-17A, IL-17F, IL-26) and transcription factors (RORγt, RORa) were overexpressed in Th17 cells. The techniques developed will make it possible to investigate the phenotypic and functional profiles of bovine Th17 cells along with their stability or plasticity.

P024

Measuring CMI responses using the PrimeFlow RNA assay; a potential new method of evaluating BVDV vaccination response

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Current methods for evaluating bovine viral diarrhea virus (BVDV) vaccination response typically rely on measurement of humoral responses as determined by neutralizing antibody titers against BVDV. While virus neutralization titers (VNT) are correlated with increased protection, research has shown that T-cell mediated immunity (CMI) is an important component of a protective response against BVDV. For example, improved protection to BVDV by modified-live viral (MLV) vaccines as compared to killed vaccines for BVDV is thought to be due to better CMI induced by MLV. The goal of this work was to evaluate the immune response in vaccinated calves using methods that quantitated both humoral and CMI responses. Classic VNT was used to measure the humoral response while a new method based on the PrimeFlow RNA assay was used for measuring CMI. PBMC from both vaccinated (MLV) and non-vaccinated calves were isolated and stimulated with BVDV. The frequency of IFN-γ, IL-2, and BVDV positive