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Johne's disease in cattle caused by *Mycobacterium avium* subsp *paratuberculosis*: an in vitro model to study early response to infection.

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Riassunto

La malattia di Johne o paratubercolosi è un'enterite cronica granulomatosa provocata dal *Mycobacterium avium* subsp *paratubercolosis* (MAP), che colpisce i ruminanti ed in particolare i bovini da latte ed ha un grande impatto economico a livello mondiale. Il MAP sembra anche avere un ruolo nella malattia umana di Crohn.

Tale patogeno è capace di sopravvivere molto bene all'interno dei macrofagi dell'ospite dove previene la loro attivazione, blocca l'acidificazione e la maturazione del fagosoma, e interferisce con la presentazione degli antigeni al sistema immunitario.

Al fine di analizzare la complessa interazione tra l'ospite e il patogeno, è stata valutata la risposta dopo 2h, 6h, e 24h di macrofagi derivati da monociti bovini (MDM), coltivati in vitro e infettati con il ceppo L1 di MAP utilizzando un approccio di RNA-Seq.

L’analisi statistica dei dati di sequenza ha mostrato un aumento del numero di geni differenzialmente espressi durante l’esperimento in risposta all’infezione. Inoltre i geni sottoespressi negli MDM infettati sono stati individuati solo a 24h post-infezione.

L’analisi dei *pathway* ha evidenziato tre *network* che sono associati alla risposta immunitaria e al processo infiammatorio. Inoltre lo studio dei geni sottoespressi a 24h ha mostrato il ruolo centrale del complemento e del complesso maggiore di istocompatibilità nella patogenesi della malattia.

Parole chiave: paratubercolosi, malattia di Johne, *Mycobacterium avium* subsp *paratubercolosis*, RNA-sequencing, trascrittomica, bovini.

Summary

Johne's disease (paratuberculosis) is a chronic granulomatous enteritis caused by *Mycobacterium avium* subsp *paratubercolosis* (MAP), affecting ruminants worldwide with a significant economic impact. MAP has also been speculated as a cause of human Crohn's disease.

MAP is a pathogen highly adapted for survival within host macrophages due to the organism's capacity to prevent macrophage activation, block phagosome acidification and maturation, and attenuate presentation of antigens to the immune system. The consequence is a very long silent infection and subclinical phases.

To decipher the complex interaction between host and MAP, the response of in vitro bovine monocyte-derived macrophages (MDM) after 2h, 6h and 24h of infection with L1 strain of MAP was explored using RNA-Seq approach.

Statistical analysis of sequence data revealed an increasing number of differentially expressed genes in MDM following infection through the three time points analysed. Furthermore down-regulated genes were only found at 24 h post-infection.

Ingenuity Pathways Analysis of differentially expressed genes showed that “cell-mediated immune response” was the most significant network related to 2hpi dataset, “immune cell trafficking” for 6hpi, and “inflammatory response” for 24hpi. Finally the analysis of down-regulated genes at 24hpi confirmed the role of complement and major histocompatibility complex (MHC) in the pathogenesis of MAP in cattle.

Keywords: Paratuberculosis, Johne’s disease, *Mycobacterium avium* subsp *paratubercolosis*, RNA-sequencing, Transcriptome, Bovine.

Preface

This thesis is submitted for the PhD degree at Università Cattolica del Sacro Cuore, (Piacenza, IT) under the Doctoral School on the Agro-Food System. The research was carried out at Parco Tecnologico Padano (Lodi, IT) in collaboration with CERZOO - Research Centre for Animal Production and Environment, Università Cattolica del Sacro Cuore, (Piacenza, IT). The project was supported by the European Small Collaborative Project (7th Framework Program) Macrophage Systems biology applied to disease control coordinated by Dr. John L. Williams. During the period of PhD a short research experience, working on bovine induced pluripotent stem cells (iPS), was carried out at the Research Division of Developmental Biology of Roslin Institute, University of Edinburgh.

The main objective of this thesis was to better understand the interactions between bovine macrophages and *Mycobacterium avium* subsp *paratubercolosis* and the genes involved in the mechanisms using the innovative methodology for RNA profiling, known as RNA-seq. Macrophages are involved in a number of key physiological processes and complex responses

such as inflammatory, immunological, infectious diseases and iron homeostasis. The mononuclear phagocyte system consists of cells derived from progenitor cells in the bone marrow. These myeloid progenitor cells differentiate into monocytes, which enter the circulation and migrate into various tissues where they differentiate into macrophages. Primary cultures of monocyte-derived macrophages (MDMs) constitute a good model for studying the biological activities of macrophages, and are excellent candidates for a transcriptomic and proteomic approach, therefore MDM were used in this study. However the tendency of monocytes and macrophages to remain in a G0-state makes them inherently difficult to maintain in culture and eventually to genetically manipulate them.

Pluripotent embryonic stem (ES) cells offer an attractive alternative to overcome these problems. Previous works have shown that macrophages can be generated by ES cells in human and mouse. However all attempts to derive convincing ESCs in ungulates have been unsuccessful. Recently it has been shown that as a pluripotent cell differentiates into a somatic cell, the latter can also be converted back into a pluripotent state, transfecting the somatic cell with candidate genes. Therefore a

trial to get bovine iPS cells was done via transfection of fibroblasts with four genes (OCT4, KLF4, SOX2, and cMYC); however the cell lines obtained seems not fully reprogrammed. Therefore the future task will be an implementation of the protocol to obtain naïve iPS cells.

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