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Pathway analysis of differentially expressed genes in *Mycobacterium bovis* challenged bovine macrophages

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ABSTRACT

The immune signalling genes during the challenge of bovine macrophages with bacterial products derived from tuberculosis causing bacteria in cattle were investigated in the present study. An *in-vitro* cell culture model of bovine monocyte-derived macrophages were challenged to *Mycobacterium bovis*. Macrophages from healthy and already infected animals can both be fully activated during *M. bovis* infection. Analysis of mRNA abundance in peripheral blood mononuclear cells from *M. bovis* infected and non-infected cattle were performed as a controls. Cells of treatment were challenged after six days for six hours incubation at 37 °C, with 5% CO_2, to total RNA was extracted then cDNA labelling, hybridization and scanning for microarray methods have been developed for microarray based immune related gene expression analysis. The differential expressions twenty genes (*IL1, CCL3, CXCR4, TNF, TLR2, IL12, CSF3, CCR5, CCR3, MAPT, NFKB1, CCL4, IL6, IL2, IL23A, CCL20, IL8, CXCL8, TRIP10, CXCL2* and *IL1B*) implicated in *M. bovis* response were examined Agilent Bovine_GXP_8 × 60 K microarray platform. Cells of treatment were challenged after six days for six hours incubation then pathways analysis of Toll like receptor and Chemokine signalling pathway study of responsible genes in bovine tuberculosis. The PBMC from *M. bovis* infected cattle exhibit different transcriptional profiles compared with PBMC from healthy control animals in response to *M. bovis* antigen stimulation, providing evidence of a novel genes expression program due to *M. bovis* exposure. It will guide future studies, regarding the complex macrophage specific signalling pathways stimulated upon phagocytosis of *M. bovis* and role of signalling pathways in creating the host immune response to cattle tuberculosis.

1. Introduction

Bovine tuberculosis (BTB) is a zoonotic disease caused by *Mycobacterium bovis* infection, a pathogen genetically and structurally related to *Mycobacterium tuberculosis* and non-developing countries *M. bovis* is a zoonotic tuberculosis infection in cattle to animal handler and dairy farmer [1]. After mycobacteria are inhaled by the host, they are engulfed by the alveolar macrophages, which become infected, pathogenesis of human and BTB occurs in a similar way, beginning with bacterial entry to host lungs by inhalation and bacteria phagocytosis by alveolar macrophages. Establishment of a chronic infection status is accomplished due to mycobacterial virulence factors that allow it to enter and survive within the host phagocytic cells [2], higher production of nitric oxide by bovine macrophages infected with *M. avium*

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subsp paratuberculosis, macrophages involved in *M. tuberculosis* infection by collection and identification of specific glycoproteins and glycogen biomarkers [3,4]. The macrophages play an important role in tuberculosis pathogenesis, being the first defense line, the niche for the bacteria and the leading control mechanism. *M. bovis* infection is by Macrophages and they mediate the host immune response to BTB through pathogen recognition and start of an inflammatory reaction [5,6].

Mycobacterial infections are major reasons of morbidity and mortality in cattle and are also prospective zoonotic agents with effects for human health. Mycobacteria to prevent immune elimination in part through differential receptor usage and the modulation of specific cell signalling pathways [7,8]. The elucidation of cell signalling pathways detected during the immune response to bovine tuberculin and their potential roles in the immune repression detected *in-vivo* [9]. Macrophages are among the first cell types to encounter *M. bovis* following exposure and the response elicited by these cells is pivotal in determining the outcome of infection. These cells are in the first line of defence and interact with a wide range of pathogens [10]. Global gene expression and systems biology analysis of bovine monocyte derived macrophages in response to *in-vitro* challenge with *M. bovis* [11].

The access of the mycobacteria into the host is through macrophages, where the bacteria can continue a long period of time without the presence of any symptoms and the initial interaction between macrophages and mycobacteria is thought to play a key role in determining the outcome of infection [12]. The pathogenesis of BTB results from a complex interaction between the pathogen and the host [13]. The population of bacteria begins replication and escapes the infected macrophages causing illness in a small proportion of individuals. The macrophage plays a key role as an effector cell, activating innate immune responses and initiating acquired immune response. Mycobacteria have developed a variety of mechanisms in order to survive the immune response, which prevent them from being killed by their host cells [14]. The effects on the host's immune response can be related to the level of pathogenicity of mycobacterial species in different hosts. The rate of bacteria proliferation and the efficiency of phagolysosomal fusion in macrophages vary among different *M. tuberculosis* clinical isolates [15]. The initial interaction between macrophages and mycobacteria is thought to play a key role in determining the outcome of infection [16].

The immune response resulting from mycobacterial infection in both macrophages and dendritic cells have been suggested as being important in inducing. Binding to the mannose receptor has also been suggested as a possible ‘safe’ route of entry for mycobacteria that facilitates their intracellular survival [17]. The recruitment of lymphocytes to the lung and granuloma formation, thus leading to containment of the mycobacteria, due to infected macrophages are known also to secrete chemokines including *IL-8, RANTES* and *MCP-1* [18]. *M. tuberculosis* infected macrophages secrete *IL-10*, rather than *IL-12*, which could act to suppress Th1 responses and Macrophages infected with *M. tuberculosis* preferentially secrete pro-inflammatory cytokines including *TNFα, IL-1* and *IL-6*. The stimulation of macrophages by other components of the immune response, such as *IFN-γ* or *TNF-α* released by T cells, can enhance macrophage microbicidal activity and this is associated with reduced *IL-10* secretion [19].

Blood is an easily accessible clinical tissue and has been used in studying the detection and pathogenesis of varied pathological conditions in tuberculosis, gene expression profiling of PBMC is a well-documented and valid tool for studying the pathogenic mechanisms in disease processes [20]. The cell mediated immunity (CMI) is of great importance in the control of intracellular pathogens, such as *M. bovis*, involving complex interactions between macrophages and T cells, the production of *IFN-γ*, which is essential to activate macrophage microbicidal pathways [21,22]. In developing countries with no active BTB control programmes, BTB poses a very significant threat to human health, greater than 94% of the world's population live in countries in which there are no strategies in place to control *M. bovis* infections [23]. The BTB affect animals a wide series of species of both domestic and wildlife species as a zoonotic disease [24]. BTB is also a threat to public health where consumption of infected unpasteurised milk and other dairy products can be a source of human infection [25]. In developing countries animal production is facing new challenges, economic development are all contributing to the increasing demand for milk, meat, eggs and other animal products [26].

In the present study, we explored differentially expressed genes, identified through comparison of the gene expression profiles from *M. bovis* challenged MDMs and the non-challenged control MDMs at the 6 h time points, were subsequently analyzed with the Ingenuity Pathway Analysis (www.ingenuity.com) and used to reconstruct the macrophage signalling pathways underlying *M. bovis* infection. The output of this research, regarding the complex macrophage specific molecular signalling pathways stimulated upon phagocytosis of *M. bovis* and the role these signalling pathways in establishing the host immune response to BTB.

2. Material and methods

2.1. Ethics statement

All animal procedures were carried out according to the provisions of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and ethical approval (SN1/03/09/2013) for the study was obtained from the Institutional Animal Ethics Committee (IAEC).

2.2. Experimental animals and infection status

The animals were selected on the basis of the skin-fold thickness response to bovine and avian tuberculin in the single intradermal comparative tuberculin test (SICTT). All the animals were tested for their parasitic status, body temperature range along with presence of concurrent infections to rule out any abnormality before screening, Tuberculosis (Bovigam, Thermo Fisher Scientific, USA), PPD tested, *IFN-γ*, Paratuberculosis (ELISA and faecal microscopy) and Brucellosis (RBPT, STAT and ELISA). Six healthy adult female animals from each of the three groups were chosen and screened. Two breeds of cattle, Tharparkar (Indigenous breed), Vrindavani (Cross breed) was used in this study from IVRI, Izatnagar and Holstein Friesian (Exotic breed), was used herd of the Raghusha Agrotech, US Nagar, Uttarakhand, India were obtained from a TB free accredited herd and no history of tuberculosis for at least 5 years.

2.3. Specimen collection and handling

Blood was collected of each animal 6 ml in sterile heparinised bottles from jugular vein of six healthy adult female animals and kept at room temperature (15–25 °C) prior to use. After collection of blood, the vials were tightly capped and shaken gently to facilitate thorough mixing of blood with the anti-coagulant.

2.4. Culture of M. bovis

Tuberculosis is caused by one of several mycobacterial species that belong to the *Mycobacterium tuberculosis* complex (*MTC*). *M. bovis* is a slow growing aerobic bacterium and the causative agent of tuberculosis in cattle (Table 1), this strain is clinical isolate from tuberculosis lesions of a tuberculin skin test-positive cow in Uttar Pradesh, India. This isolate of *M. bovis* was supplied by ICMR-National JALMA Institute of Leprosy and Other Mycobacterial Diseases, Tajganj, Agra, Uttar Pradesh, India.