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Review on *Mycobacterium bovis* in Host Immune Response Interactions and Omics Integrative Approaches in Infection

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ABSTRACT

Bovine tuberculosis economically important disease with a wide range of host throughout globally. Mycobacterium bovis is the causative agent which transmissible in between species or within species. Among from route of transmission, aerosol inhalation is the primary one. The virulence and host factors is the key to determine infections and broadly to control the mode of transmission. Today infectious disease easily identified and characterized. Similarly, antibiotics and vaccines discovery simplified based on omics analysis and development of bioinformatics tools. So, Transcriptome was considered as the most informative assay in order to start with functional genomics to explore the relationship between genotype and phenotype of an individual. Transcriptome analyses were used to understand the mechanisms of pathogenesis of a disease and genes responsible for protective immune responses. Genes associated with specific diseases known as biomarkers can be determined. Using proteomics protein expression can be described in 3D structure and protein functions characterize by the role of proteins, glycoprotein and how proteins were expressed and the overall proteome at the level of macrophages, DC and lymphocytes cells or tissues were affected in response to M. bovis infection.

Keywords: *M. bovis, Infection, Host Immune Response, Omics Integrative Approaches.*

INTRODUCTION

Mycobacterium bovis is a member of Mycobacterium tuberculosis complex (MTC). This pathogen is well-known by fast transmissible disease distributed throughout worldwide. It's the causative agent of Bovine tuberculosis (BTB) mainly characterized by aerobic, non-motile, non-spore forming, slow growing, rod shaped gram positive and acid fast bacilli. BTB is the top of economically significant disease of public health and livestock especially countries under developed and developing poses potential zoonotic risk or losses due to the productivity of infected animals, condemnation of carcasses at slaughter houses, abattoirs and an increased risk of restrictions on animal trade. The anatomical distribution of lesions in cattle depends on the route of infection mainly inhalation is the main mode where nasopharynx and lower respiratory tract are

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mostly affected. Pulmonary tuberculosis transmitted by lympho-haematogenous of infected macrophages, moist coughing up, sneezing, contaminated milk, eating raw meat, urine, feaces and swallowing contaminated sputum [1,2].

After *M. bovis* infects the prone host, it's usually characterized by a tubercle or tuberculoid granuloma which was seen as typical lesion. Tuberculoid granuloma is a morphologically unique lesion, modified into epithelial-like (epithelioid) macrophage. The tuberculoid granuloma has a central region of caseous necrosis enclosed by epithelioid macrophages and multinucleated giant cells with an outermost zone containing increasing numbers of lymphocytes and occasionally plasma cells. Multinucleated giant cells formed via a distinct macrophage differentiation pathway induced by persistent inflammation. The entire masses of necrotic tissue and inflammatory cell infiltrate encased within a variably thick fibrous capsule. Tubercle occurs as a heterogenous in nature which primarily used as a niche for pathogen persistence. Tuberculoid granuloma actively known by dynamic in duration of growing and shrinking time, where key interactions between host and pathogen determine disease control, dissemination, bacterial replication, killing or latency. Individual granulomas within the same host and tissue can have various effects indicating that local rather than systemic responses to determine the outcome [3,4].

Transcriptional gene expression analysis used to describe the repertoire of genes expressed in host-pathogen interactions in all gene transcripts in a particular cell. The regulation of genes, biological functioning and detection of genes those are important in diseases or production traits to be identified. The differential expression analysis was used to analyze expression data to compare expression levels between healthy vs diseased state. Currently RNA-sequencing (RNA-Seq) studied on the transcriptome of individual animal in order to detect novel transcripts, candidate genes and genetic variants. Single-cell transcriptome is the one ways of a novel method analysis which gives a deep-sequencing insight into the cell's gene transcription [5]. Prokaryotic proteins analysis was challenging because of their hydrophobicity and hydrophilicity properties. Proteomics is a key for early disease diagnosis, prognosis and drug development as target molecules and responsible for characterization of proteome expression, structure, functions, interactions and modifications of proteins at any point. The proteome fluctuates from time to time, cell to cell in response to external stimuli. Proteomics in case of a multicellular organism cells, its very complex due to presence of post-translational modifications which arise at various sites [6].

According to (World health organization (WHO)) [7], World organization for animal health (OIE), food and agriculture organization of the united nations (FAO), reports in 2017, high numbers of cases about 147,000 and 12,500 deaths were recorded due to zoonotic tuberculosis. Therefore, studies rise out there possible reasons were uncontrolled association between human and domestic animals as well as wide ranges of animals grazing on similar pastures were identified as a major gap. Therefore, the objective of this review was to identify the role of *M. bovis* in host immune response interactions and omics integrative approaches in infection.

Overview of *M. bovis* in host immune response interactions and Omics interactive approaches

Virulence factors

Cell Envelope

The cell wall of *M. bovis* consists of biomolecular components comprising hydrophobic mycolic acids which are responsible for the acid-fast properties, arabinogalactan, peptidoglycans, mannose-containing biomolecules, mannose-capped lipoarabinomannan (LAM) and lipomannan (LM). From all cell wall components LM and LAM were the major glycolipids involving in pathogenesis by modulation of host immune functions. *M. bovis* invasion starts with host recognition of outer surface molecules that bind to the host-pathogen associated multiple cell surface receptors by TLR, mannose and c-type lectin family to gain entry to macrophage. *M. bovis* attain in disease causing involves numerous mechanisms to sustain in host by colonization and replication. Virulence factors genes or cellular components which lead *M. bovis* survive in host. Deletion or loss of any particular gene impairs the agent growth in the host. Virulence factors detaily known by genomic, biochemical and functional analysis of M. bovis [8,9].

PE/PPE Family Proteins

PE and PPE proteins named after the conserved proline (P) and glutamic acid (E) residues existing in their N-terminal sequences. Three covalently attached molecules to a layer of arabinogalactan, peptidoglycan and large amount of long-chain (C60-C90) fatty acids known as mycolic acids makes mycobacterial cell wall was unique. The mycolic acids layer forms the inner leaflet of the mycobacterial membrane while various complex lipids including glycolipids form the outer part of membrane which together forms a thick hydrophobic barrier difficult to penetration. There are an outer membrane lipids has a role in virulence factors like Pro-Glu (PE)/Pro-Pro-Glu (PPE) family proteins which exhibit outer membrane localization and provide a newly-identified means for nutrient and protein transport across hydrophobic barrier. Phthiocerol Dimycocerosates and Phenolic glycolipids both of them structurally related complex lipids present

in the mycobacterial outer membrane which have critical virulence importance. PDIMs are methyl-branched fatty acid-containing lipids present in *M. bovis*, contribute to host cell for necrosis and independently not properly functions virulence factors mediated with *EsxA* secretion system-1 (*ESX-1*), a type VII secretion system [10,11].

Secretion system

ESX secretion systems was desirable for transport of virulence factors via thick mycobacterial cell envelope comprises an inner phospholipid bilayer and an outer membrane (mycomembrane) known by mycolic acids into host cells together with immunodominant co-secreted effectors, EsxA (*ESAT-6*) and *EsxB* (*CFP-10*). Three types of *ESX* secretion systems were identified in mycobacteria namely ESX-1, *ESX-3* and *ESX-5* which required for full virulence. ESX-1 secretion system within the region of difference 1 (*RD1*) was discovered by comparative genomics between virulent M. bovis. *BCG* vaccine strains lack the overlapping portions of genomic *RD1* encoding the *ESX-1* secretion system. The ESX-1 secretion system was a protein complex localized in the plasma membrane. The *ESX-1* secretion mechanism nearly resemble to ESX-5 system based on cryoelectron microscope revealed a hexameric organization. The main effector protein ESAT-6 was secreted by *ESX-1* as a heterodimer together with *EsxB/CFP-10* (10 kDa of similar amount. The secretion of *ESAT-6/CFP-10* was dependent on *ESX-1*-associated proteins. There are various *ESX-1*- associated proteins encoded by the *ESX-1* locus. *EspB* was substrate of *ESX-1* forms folds similar to *ESX* effector protein to PE/PPE proteins, containing a similar N-terminal region. *EsxA* has the ability to disrupt cell membranes, potentially catalyzing the *ESX-1*. *ESAT-6* was important in modulating host inflammatory responses by manipulating several intracellular signaling pathways in macrophages, T-cells and epithelial cells. During early infection, *ESAT-6* induced differentiation of M1 to promote pro-inflammatory and M2 macrophages to maintain infection at later persistent phases [10, 11].

Lipid metabolism

Lipid-laden foam cells were pathological observations for a number of infectious diseases by fat deposition into cytoplasmic lipid droplets (*LDs*). The concentration of lipids within the cytoplasm forming an organelle known as a LD also called an adiposome. LDs were complex organelles composed of a triglyceride, cholesteryl ester core, a surrounding monolayer of phospholipid, cholesterol and a varied array of associated proteins in cell metabolism and signaling [12].

LDs proteins that share sequence similarities known as polyacyltrehalose (PAT) family of proteins. PAT protein family includes perilipin, adipose differentiation-related protein (ADRP) and tail-interacting protein of 47 kDa [13]. Proteins from PAT family existed at surface of LDs used as markers of LDs for biogenesis in cells. Perilipin is the most abundant protein on the adipocyte LD which modulates lipase functions. ADRP involved in the regulation of LD accumulation in different cells. The accumulated lipids in LDs involved in biosynthesis, transport and regulation of cellular lipid metabolism. Proteins involved in membrane and vesicular transport used for cell signaling and inflammatory mediator production including eicosanoid-forming enzymes, phospholipases and protein kinases localized to LDs in different cells [12].

LDs formed during infection which activates various intracellular signaling pathways to culminate in lipids and proteins formation. LPS stimulates the formation of LDs via *TLR4*-dependent signaling by limiting the production of inflammatory mediators such as *PAF* and *MCP-1/CCL2* amplifies the response. *LDs* formation was dependent on *ADRP* synthesis and fatty acid synthase (FAS) activity as well as microtubules directing the droplet assembly [13, 14].

The presence of foamy lipid-laden differentiated cells observed in infections and LD biogenesis detected after a short time stimulation of direct interaction between pathogen and host cells. Cytokines and chemokines partly mediated by the phagocytosis of apoptotic neutrophils and macrophages. Platelet activating factor (*PAF*) acts as intracellular binding sites to induce cell activation. PPAR-γ expression increased due to infection which enhanced by *ADRP* and *FAS* expression. *PPAR-γ* is a lipid activated nuclear receptor which is a key for transcriptional regulator of cell differentiation, inflammation, lipid metabolism in macrophages and dendritic cells. PPAR transcription factor directly regulates the expression of several genes participating in fatty acid uptake, lipid storage and the inflammatory response by binding to specific DNA response elements in target genes as heterodimers with the retinoid x receptors by *FAS* and *ADRP*. Increased expression of scavenger receptors by macrophage receptor with collagenous structure and *CD36* leading to increased uptake and accumulation of host-derived oxidized lipids in the infected cells [13, 14].

Host Immune Response factors

Innate immune responses

Macrophages

Macrophages are a primary cellular niche during infection and eliminate *M. bovis* via multiple mechanisms including production of O_2, N_2 components and cytokines, phagosome acidification (phagocytosis), autophagy by alveolar macrophages and antimicrobial

peptide production enhanced by vitamin D treatment. The recognition of pathogen- associated molecular patterns from glycolipids, lipoproteins and carbohydrates set by macrophage PRRs (*TLRs*, *NLRs* and *CLRs*) induce a coordinated network signaling pathways in gene expression at various steps of infection. M1 macrophage expresses inducible nitric oxide synthase (*iNOS*) which is the primary marker that confirms the response of a specific disease while M2 macrophages (*M2a*, *M2b*, and *M2c*) characterized by the presence of arginase. Macrophages in lymph nodes can be spinal or sub-capsular sinusoidal which capture antigens and present them to B cells. Macrophages express many diverse receptors that recognize mycobacteria, mannose receptors bind mannosylated glycoproteins and scavenger receptor binds low density lipoproteins [15-18].

When antigens bind to macrophages lead to phagocytosis with lysosome fusion to create a phagolysosome. Mycobacterial breakdown products stimulate interleukin-1 receptor-associated kinase and combined with signaling from MyD88 leads to transcription of NF-κB. TLRs and NLR on APCs were essential for recognition. TLR2, TLR4, TLR9 and NLR-2 bind with mycobacteria to initiate immune activation. Recognition of mycobacteria via TLRs on APC leads to Myd88 activation and binding to *IRAK*. The effect activates *NF-κB* to signal cytokine production. Activated M1 macrophages are effectors of the host response produce immune-stimulatory cytokines (*IFNγ, TNFα* and granulocyte macrophage colony-stimulating factor). Activated M2 macrophages are poor APCs and suppressors of Th1 responses induced by *IL-4*, *IL-13*, *IL-10* and TGF_{β}. A population of macrophages suppresses T-cell responses via secretion *of IL-10*. *TGF-β* exists as a novel class of immune cells used to regulate infection associated with *M. bovis* and characterized by a broad spectrum of transformed macrophages like multinucleated giant cells, epithelioid cells and foam cells granulomas. Foamy macrophages have ability to mediate phagocytosis accompanied by reduced antigen processing capacity and increased secretion of *TGF-β* induced by mycolic acids, lipopeptides and *ESAT-6*. *ESAT-6* induces metabolic flux perturbations to drive foamy macrophage differentiation. Increased expression of *IL-10* attempts to down-regulate pro-inflammatory immune response and decreasing the ability of macrophages to restrict growth [3, 19-21].

M1 and M2 macrophages polarization balanced by host to control infections. Inflammatory markers regulate the formation of granuloma and determines prognosis. Infected foamy macrophages are packed with host lipid which is consumed by *M. bovis* via aerobic glycolysis. Foamy macrophages leave the original granuloma disseminated to secondary granuloma formation. Granuloma includes necrotic areas known as caseum made up of neutrophils, *NK* cells, *DCs*, B and T-cells. This structure was lined with epithelial cells, histiocytes (mature monocytes) and lymphocyte plays a key role in granuloma formation. Histiocytes activated by T-lymphocytes which secrete IFN-γ infected macrophages become necrosis then persistence of chronic infection occurs. The primary lesion can spread to the local lymph system undergoing latency and calcifications [22].

Dendritic cells (DCs)

DCs originated from stem cells precursors in the bone marrow used as bridging or connect innate and adaptive immunity arms of immune system. *DCs* are one of antigen presenting cells which capable to activate various cells of immune system like *NK* cells, *γδ* Tcells and naive T-lymphocytes. *DCs* were known by numerous immune cells to regulate the development of immune responses by maintaining immune tolerances. The monocytes serve as a primary antigen-presenting DC within peripheral tissues during steady-state and inflammation. The subtype of DCs, (monocyte-derived DC) generated from peripheral blood mononuclear cells of infected tissues. After the intake of pathogen, antigens were processed and expressed by major histocompatibility complex (MHC) type II. *DCs* produce type I and III interferons necessary for an immediate host defense against pathogen, recognize and take up foreign antigens then migrate to lymph nodes to present processed antigen to T cells [21,23,24]. DCs present antigens to T cells and promote T-cell homing to the lung through their induction of chemokine receptor *CCR4*. During ligation of *DC* with *M. bovis* mannose-capped lipoarabinomannan induces the production of anti-inflammatory cytokine IL-10 which used for impairs *DC* maturation and the expression of co-stimulatory molecules. Both *DC* and macrophages phagocytosed *M. bovis*. Macrophages can able to kill more organisms than *DC*. Cytokine secretion was higher in *M. bovis*-infected *DC* but lesser amounts found in macrophages due to high number of mycobacteria exist in the *DC*. *IL-12* secretion by *DC* was essential to activate *NK* cells to release *IFN-γ*. *TNF-α* is secreted by both *DC* and macrophages make conducive by amplifying antigens for killing. When DC infected by the pathogen like *M. bovis*, develops a mechanism which capable to up-regulate the expression of MHC II and CD80. *DC* is more efficient at stimulating T-cell responses and macrophages are effective at killing mycobacteria [21,23].

Neutrophils

Neutrophils were abundant cells to infiltrate the lungs after *M. bovis* appearing in bronchoalveolar lavage and involved in the initiation of adaptive immunity critical for granuloma

cavitation during infection. *NK* cells in combination with macrophages and DCs responsible for control infections in early stages and influence adaptive immune responses against pathogen attack by applying B and T-lymphocytes. Respiratory bursts such as *elastase*, collagenase and myeloperoxidase released by neutrophils broadly damage the antigen and host cells. Neutrophils release enzymes lead to the destruction of pulmonary parenchyma like *arginase*, matrix metalloproteinase-9 (*MMP-9*) and *gelatinase* shared by other innate immune cells and epithelial cells. Apoptotic neutrophils and purified neutrophil granules contain active antimicrobial peptides taken up by macrophages which lead to inhibition of mycobacterial replication.

The autophagy-related gene 5 (*Atg5*) is dispensable in alveolar macrophages during infection. *M. bovis* induces neutrophil necrosis and prevents apoptosis on region of RD-1-encoded virulence factors. *ESAT-6* protein secreted by a type *VII* secretion system (*ESX*) encoded by *RD1* in *M. bovis* induce an intracellular Ca^{2+} followed by necrosis and formation of neutrophil extracellular traps [21, 24].

Natural killer cells

NK cells are innate effectors preformed granular innate by lymphocytes of lytic proteins having perforin and granulysin which released for recognition of target cells to possess potent cytolytic capacity. Granule components directly kill extracellular bacilli and reduce the viability of intracellular mycobacteria. When *NK* cells activated, the development of immune response activated mainly by production of cytotoxicity and cytokines. Mycolic acids of *M. bovis* were direct ligands for natural cytotoxicity receptor on *NK* cells. *NK* cells produce *IFN-γ* and *IL-22* which inhibit *M. bovis* intracellular growth indirectly by enhancing phagolysosomal fusion, immune stimulation and activation of macrophage. *NK* cells promote *γδ T*-cell proliferation by producing *CD54*, *TNFα*, *GM-CSF* and *IL-12*. Both early innate immune and *NK* cells functions in mature granulomatous lesions in the lungs of *M. bovis* infected host [21,25].

Adaptive immune response

After *M. bovis* penetrating in the host, innate immune system actively initiated and starts its role in first line of defense. *DCs* act as *APCs* to T-cells in lymph nodes. Adaptive immune system is activated after innate immune response, antibody production and killer T-cells activated to attack the pathogens. Adaptive immunity can be *CMI* and humoral responses. The *CMI* includes T-lymphocytes activation and effector mechanisms while humoral responses involve B-lymphocytes maturation and antibody production. Both responses stand together to achieve their mechanisms of diseases action. T-lymphocytes are required for antibody maturation, isotype switching and memory. B-lymphocytes act as antigen presenting cells by activating T-lymphocytes. For intracellular infections, the primary protective immune response against MTC infections is cell mediated rather than antibody mediated. *M. bovis* resides inside the macrophage which is relatively resistant to mechanisms efficiently eliminate other phagocytosed bacteria. This mechanism was due to the ability of the bacilli to hinder macrophage activation by IFN-γ and *IL-12*. Deficiencies in IL-12, IFN-γ or their receptors make the individual more susceptible to mycobacterial infections [25,26].

Cellular immune response

Macrophages are a key cell in the main host and a crucial effector cell to control and killing the mycobacteria via lysosomal enzymes, reactive oxygen or nitrogen intermediates. When the pathogen reaches in the lymph nodes, the naive T-lymphocytes activated by APCs mostly by DCs migrate from the alveolar interstitium to the lymph nodes. T-lymphocytes performed by APCs using the MHC type II receptors while after presentation to the $CD8^+$ T-cells carried out by MHC type I. Activated $CD4^+$ effectors T-lymphocytes migrate from lymph node through circulatory torrent and engaged into the primary focus of infection to contribute in the inflammatory response. *DCs* exposed to MTC produce *IL-12* which is responsible for the $CD4^+$ T-cells differentiation into Th1 cells. The main function of Th1 cells is the production of cytokines like *IL-2* which participates in activation and proliferation of T-lymphocytes. It also produces *IFNγ* and *TNFα* that activate macrophages. Induction of a Th1-type immune response provides the host to greatest protective capacity [25].

Humoral immune response

Humoral immunity is the mechanisms of host defense against from microorganism's invasion. Several factors limit the host humeral response such as environmental conditions and genetic factors mentioned on immunoglobulin response. The immunoglobulins secreted by B-cells. During initial steps of infection, primarily antibodies standing alone or with various cytokines fight against entry of mycobacteria. Antibodies used in two ways first in clinical management and by actively protecting infectious disease or as serologic diagnostic tools. Immunity usually supported by antibodies through various mechanisms including neutralizing toxins, promotion in release of cytokines, antibody-dependent cytotoxicity, complement activation, opsonization and enhancing antigen presentation [25].

Omics Integrative Approaches in Infection

Gene expression constantly changes when the hosts become sick, adaptation, aging and toxicity. Whole genome is a genetic blue print of an individual is almost

static but numerous levels of gene expression reformed to operate in a complex organism which actively regulated, spatially determined in response to environmental alarms and growth situations. Gene expression signatures like transcriptome and proteome used to explore and determine underlying molecular and cellular processes. RNA sequencing and shotgun proteomics technologies open new access to make probing transcript and protein expression at an unexpected depth and coverage. The rapid change of host cell or tissue can be determined by measuring its part of expressed transcriptome and expressed protein set from proteome. In other ways post-transcriptional and posttranslational also used to determine regulations of gene expression processed in the host. Gene expression regulation contain alternative splicing, editing of expressed transcripts and post-translational addition of covalent modifications to proteins [27].

Various isoforms or proteoforms having dissimilar structural and functional traits may originate from a single gene. The diverse biological macro-molecules exist can be reveal a surprising number of opportunities in a molecular pattern. Deciphering the gene expression patterns particularly existed with a given biological state in order to understand cellular processes and disease state [27]. DNA methylation and histone modification possess a vital role in gene expression. Methylation of CpG islands represses the initiation of transcription in somatic cells not in reproductive cells. CpG sites were unevenly distributed into CpG islands which were usually located in the promoter regions. A small region within CpG islands in the promoter regions was normally methylated and associated with gene silencing by transcriptional regulation. A genome-wide association study was confirmed transcription factor binding can be strongly influenced by methylation of CpG sites within their recognition sequences [28].

Transcriptomics

Transcriptome was considered as the most informative assay in order to start with functional genomics to explore the relationship between genotype and phenotype of an individual. Transcriptome analyses were used to understand the mechanisms of pathogenesis of a disease and genes responsible for protective immune responses. Genes associated with specific diseases known as biomarkers. After the biomarkers identified with genes it was provide evidence on disease diagnosis, clinical status determination and disease progression. Transcriptome profile delivers immediate expressed genes associated with particular phenotypes. The integration of genomic profiles signatures related to innate and adaptive immune response was very useful for BTB to distinguish between active and latent tuberculosis which signifying genomic data at the nucleotide information flow to search for gold biomarkers. Using comparative omics technologies *M. bovis* and *M. tuberculosis* compared depending on their species differences in response to infection of the host and the macrophage gene expression shared 99.5% homology. DNA microarrays used for rapid and direct detection of *M. bovis* in bovine milk based on mtp40 and pncA sequences. DNA detection limited by DNA microarray was 50 fg or 5 tubercle bacilli [29-31]. PCR and DNA microarrays combination increases the detection of infected bovines with *M. bovis* and reduced the number of false positive animals. Bovine monocytes derived from macrophages were challenged with *M. bovis* and gene expression signatures were determined [29].

Single gene signature profiles, the dynamics of mRNA transcripts in BTB, innate cytokine pattern induced after infection of alveolar macrophage with *M. bovis* were seen in these cells. DNA microarrays, NGS and RNA-seq discovered alveolar macrophage infected with tubercle bacilli of host immunomodulatory response. In order to transcript a gene into mRNA and translate mRNA into a protein various types of RNAs were involved [29]. The majority of known T-cell antigen-encoding genes, conserved as essential genes. The epitope-encoding regions within these genes, the most conserved regions of these or any genes. T-cell antigens confirmed by a comparative analysis of sequenced *M. bovis* and smooth tubercule bacillus strains. *PE-PPE* genes constitute a highly repetitive antigenic gene family typically not captured by shortread sequencing. The success of *M. bovis* in causing disease involves various mechanisms that enable colonization, replication and survival in their host. The virulence factors are typically defined as bacterial genes or cellular components that enable their overall survival in the host [32].

Dual RNA sequencing allows simultaneous transcript profiling of a pathogen and its infected tissue host. When acute inflammatory initiated, germ line-encoded PRRs present in distinct cellular compartments respond to signs of infection. After activation, PRRs trigger signaling link with transcription factors [33]. Mobilization of transcription factors leads to rapid, dynamic and temporally regulated changes in the expression of hundreds of genes involved in antimicrobial defense, phagocytosis, cell migration, tissue repair and in regulation of adaptive immunity. Multiple genes within distinct functional categories can be coordinately and temporally regulated by transcriptional on and off switches that account for the specificity of gene expression in response to external stimuli. Multiple layers of regulation-including chromatin state, histone or DNA modifications and the basal transcription machinery-collaborate to control

pathogen-induced or danger signal-induced gene expression programmes, which vary depending on the cell lineage involved and the specific signal that is encountered. For *M. bovis* infection in host gene based network and pathway detection methods analyzed under a statistical framework based on gwinteR tool software to integrate transcriptomics data [33,34].

Transcription is the first step, well examined area in studies of innate immunity, proper regulation of immune genes involves an excess of additional post-transcriptional checkpoints. These occur at the level of mRNA splicing, mRNA polyadenylation, mRNA stability and protein translation. These mechanisms are important for modulating the strength and duration of the response and for turning the system off in timely and efficient manner. The role of posttranscriptional regulation in controlling gene expression in macrophages and other innate immune cells is very important. Analysis of the host and pathogen transcriptome is highly informative as well as used to identify genes involved in pathogenesis that other methods have failed to uncover [33]. RNA-Sequence analysis was used to determine the response of bovine monocyte-derived macrophages (*bMDM*) with two strains of *M. bovis* infection, *AF2122* and *G18*. Comparison of transcriptional levels helps to know differentially expressed genes in response for every *M. bovis* infection. With respect to hpi analysis, at 6 hpi highest number of differentially expressed genes was observed in response to AF2122 than G18 but at 2 hpi, there was greatest differentially expressed in the G18 only 10.1% differentially expressed genes being down regulated. Common gene expression response was primarily affected by infection with AF2122 than G18 which was confirmed at 6 hpi the expression was only 86 (12.3%) from 702 common response genes visible variation arisen in response to G18 than AF2122 [35]. The temporal overlap difference was observed between differentially expressed genes at 24 hrs and 48 hrs time points. About 38.9% genes were differentially expressed at the two time points. Only 12 to 14.4% of genes overlapped between the early and late time points compared with differentially expressed genes 21.6% between the 2-hrs and 6-hrs time points. Functional transcriptional response of *bMDM* to *M. bovis* infection was analyzed by online database for annotation, visualization and integrated discovery (DAVID). DAVID mainly used to functional annotation in order to interrogate differentially expressed genes. Database of bioinformatics was located overrepresented via gene ontology and Kyoto Encyclopedia of Genes and Genomes for the pathways [35].

Proteomics

Proteomics is the applications of tools to describe protein expression into (3D) structure and protein functions in BTB which is characterize the role of proteins, glycoprotein, how proteins expressed and the overall proteome at the level of macrophages, DC and lymphocytes cells or tissues were affected in response to *M. bovis* infection. Protein-protein interactions are usually a vital for regulation of physiological processes and pathogenesis within the host. Protein phosphorylation acts as a reversible molecular switch which provides a mechanism for the control of protein function during all cellular processes and is essential for rapid responses of cells to internal and external signals. Kinases and their substrates form dynamic complexes and temporal information processing networks which facilitate cell to cell communication and cellular responses to changing environmental conditions. Antigenic targets of T-cells in BTB, subset of T-cells and their interactions with infected macrophages by *M. bovis* can help for better disease control [29]. Phosphorylation-based signaling bears positive impact to innate and adaptive immunity in defense mechanisms of the mycobacteria. The covalent attachment of phosphate groups from adenosine triphosphate (ATP) to proteins of serine, threonine and tyrosine residues achieved by protein kinases and the phosphate groups attached at these residues removed by protein phosphatases. In most cells about two-thirds of individual proteins phosphorylated at multiple sites. The function of protein regulated by phosphorylation via conformational changes directly modulating enzymatic activity or offer by a docking site between molecular protein interactions or within molecular protein interactions. The immune system actions like differentiation, cytokine/chemokine production, inflammation and pathogen killing controlled by protein phosphorylation and later by the corresponding protein kinases. In innate immune system, pathogen-associated molecular patterns are recognized by specific PRRs to activate pro-inflammatory and antimicrobial responses [36,37].

When *TLR* proteins stimulated following the activation of many kinases such as interleukin-1 receptor-associated kinases (*IRAK1*), mitogen-activated protein kinases (*MAPKs, MAP3K7/TAK1*, *p38-alpha* and *JNK*) and *IκB* kinase (*IKK*) as well as following phosphorylation of downstream targets like activator protein 1 (*AP-1*) and nuclear factor-*κB* (Nf-κb) used as master transcriptional regulators during the induction of pro-inflammatory and anti-apoptotic mediators outcomes occurred. Deregulation of such signaling processes related with inflammatory diseases, autoimmunity and pathogenesis of infections. During infection pathogens achieve host signaling pathways linked with major processes like membrane and cytoskeleton dynamics, autophagy, vesicle trafficking, cell death, inflammation and immunity. Pathogens have evolved various mechanisms by production of specific toxins or virulence factors by controlling signaling

pathways enables to adherence, survival, replication or dissemination pathogens [36,37].

Cellular proteome is a complex system of structural and regulatory networks relies on information to attain the dynamic needs of the cell. The cellular proteome is profiled following the complete lysis of cell by mechanical (grinding) or chemical (detergents) disruption, reduction, alkylation and digestion of the proteins to peptides finally measured and quantified by LC-MS and mapped to the provided protein sequence databases. Another proteome profiling strategies include fractionation of the cell into specific compartments to determine localization patterns. As cellular proteome fluctuates during host-pathogen interactions, the cell modulates its response to the environment, secretes proteins into the host (effector proteins), produces virulence factors and adapts biological processes to promote survival [38,39]. Proteomics deals with the qualitative information on proteins (identification, distribution, posttranslational modifications, interactions, structure and function) and quantitative information like abundance, distribution within different localizations and temporal changes in abundance due to synthesis and degradation or both. Knowing about host-pathogen proteomic interactions enables to identify the genome and measure the required proteins from the causative agent in a quantitative manner using proteomic discovery techniques. Currently due to enormously emerging of bioinformatics tools and application simplifies the determination of multiple species in a single sample useful for analysis between a host and pathogen interaction to identify the invasion and evasion of host at the same time. The high-resolution of MS-based proteomics workflows can be bottom-up, top-down and targeted proteomics. The bottom-up proteomics discovery relies on sequence specific enzymatic digestion of proteins into peptides prior to separation by liquid chromatography and measurement by mass spectrometry. In top-down proteomics detection depends on intact proteins for the identification of protein complexes. While targeted proteomics is similar to bottom-up with the measurement of digested proteins in the mass spectrometer which is optimal for detection as well as accurate quantification on a set of predefined peptides in a complex mixture and used for biomarker detection and development [40, 41]. Discovery-based bottom-up proteomics begins with sample preparation and collection, followed by protein extraction and enzymatic digestion of proteins into peptides. Peptides are purified on *C18* columns and separated by high-performance liquid chromatography [42-47].

CONCLUSION

M. bovis is the etiological agent of Bovine tuberculosis The impact of the disease economically accountable for various public health and livestock risks by directly or indirectly means especially in those lower income countries. Human, domestic animals and wild animals are highly vulnerable or susceptible for infections of BTB. Among from ways of transmission inhalation of aerosols is leading route of transmission. Many virulence factors make *M. bovis* to begin invasion with host recognition of outer multiple cell surface molecules of receptors by *TLR*, mannose and c-type lectin family and bind on to host that enable to gain entry in to macrophage. *M. bovis* attain in disease by causing numerous mechanisms to sustain in host by colonization and replication. Virulence factors genes or cellular components which lead *M. bovis* survive in host. Deletion or loss of any particular gene impairs the agent growth in the host. Virulence factors detail known by genomic, biochemical and functional analysis of *M. bovis*. From host factors the first line of defense mechanisms and second line defenses try to eliminate *M. bovis* developing via multiple mechanisms including production of O_2, N_2 components and cytokines, phagosome acidification (phagocytosis), autophagy by alveolar macrophages and antimicrobial peptide production enhanced by vitamin D treatment. If the defense mechanisms failed the *M. bovis* develop diseases. Gene expression signatures like transcriptome and proteome used to explore and determine underlying molecular and cellular processes. RNA sequencing and mass spectrometery proteomics technologies open new access to make probing transcript and protein expression at an unexpected depth and coverage. The rapid change of host cell or tissue can be determined by measuring its part of expressed portion of transcriptome and expressed protein set from proteome.

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