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Evaluation of the expression of cytokines and chemokines in macrophages in response to rifampin-monoresistant *Mycobacterium tuberculosis* and H37Rv strain

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ABSTRACT

Macrophages are the primary phagocytes in the lungs and a part of the host defense system against *Mycobacterium tuberculosis* (*Mtb*), involved in the primary immune response. While several studies have assessed the effects of resistance to rifampin on *Mtb* physiology, the consequences of mutations in genes encoding the beta subunit of RNA polymerase (*rpoB*) for host-pathogen interactions remain poorly understood. In this study, rifampin-monoresistant (RMR) *Mtb* and H37Rv strains were used to infect the THP-1-derived macrophages. Real-time quantitative reverse transcription PCR assay was carried out to determine mRNA expression in 84 cytokine and chemokine genes. Production of specific cytokines and chemokines was measured by ELISA assay. In conclusion, the current study shed more light on the fitness cost of RMR strain and the potential effects of *rpoB* gene mutations on *Mtb*-host interactions. These results initially demonstrate that the *Mtb* carrying the *rpoB*-S450L can modulate macrophage responses to mediate bacterial survival.

1. Introduction

Tuberculosis (TB) is a major global health threat, accounting for 10.4 million diseases and more than 1.7 million deaths annually around the world [1]. Interactions between *Mycobacterium tuberculosis* (*Mtb*) as an airborne bacterium and alveolar macrophages are significant in terms of not only pathogenesis, but also the main immune response to *Mtb* [2,3]. Induction of immune responses depends on the complexity of interactions between the host and bacteria, causing variations in the immunopathology of diseases [4].

Macrophages are the primary phagocytes in the lungs and a part of the host defense system against *Mtb*, involved in the primary immune response [5]. The intracellular signaling cascade can lead to the production of proinflammatory and anti-inflammatory cytokines. Macrophages are capable of inhibiting, enhancing, or establishing a balance in the production of cytokines and chemokines. Among these products, interleukin-1α (IL-1α), IL-1β, tumor necrosis factor-alpha (TNF-α), IL-12 family, IL-6, and IL-10 are the major cytokines, produced by macrophages against *Mtb* [6]. Several studies have shown that different *Mtb* strains can induce a variety of host responses in macrophages [7,8]. The THP-1-derived macrophages is a relatively similar model to human macrophages [9]. Indeed, THP-1-derived macrophages have shown similar functionality to macrophages in terms of immune responses to *Mtb* [10].

According to the literature, TB is more persistent in individuals with multidrug-resistant TB (MDR-TB), compared to those infected with a susceptible bacterium. Based on several studies, in immunocompromised individuals with MDR-TB, the host immune system is significantly suppressed, and levels of anti-inflammatory cytokines, such as transforming growth factor-β (TGF-β) and IL-10, are increased [11,12]. However, this question arises as to whether mutations in MDR bacteria can cause immunological responses. Since MDR bacteria have different mutations, the answer to this question is very complicated. To address this issue, examination of a monoresistant bacterium, such as rifampin-monoresistant (RMR) *Mtb*, can be helpful [13].

An RMR strain is characterized by a mutation in the rifampicin-resistance determining region (RRDR), including mutations in the 81-bp core region of the gene, encoding RNA polymerase β subunit (*rpoB* gene) [14,15]. As confirmed in multiple studies, the RMR strain is an appropriate model for evaluating the mechanisms of antibiotic resistance and a proxy for MDR-TB [16,17]. While several studies have assessed the impact of rifampin resistance on *Mtb* physiology [18],

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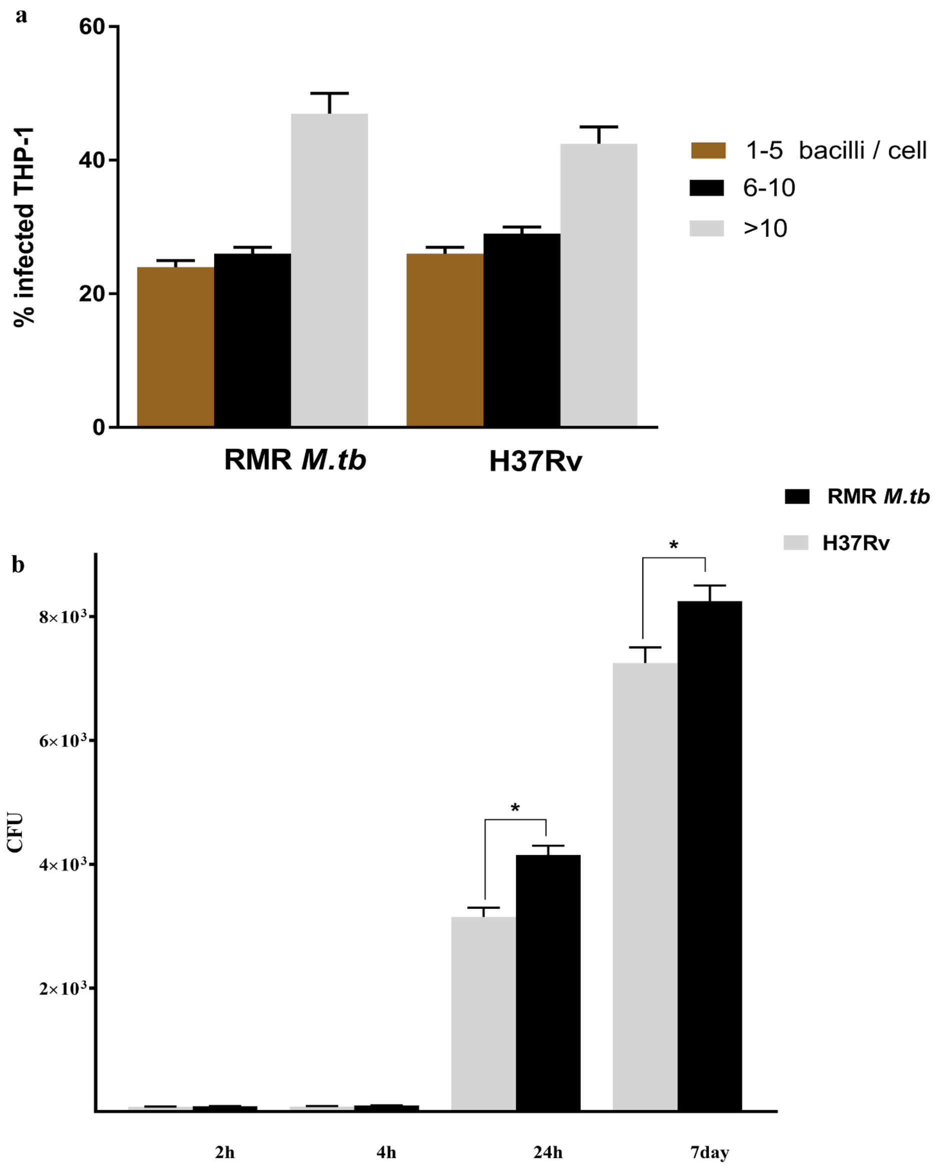

Fig. 1. (a) Graph shows the percentage of infected cell by two strains (RMR and H37Rv); intracellular internalization index was classified into 1–5 bacilli, 6–10 bacilli, and more that 10 bacilli per cell; (b) intracellular growth of different studied strains in THP-1-derived macrophages.

consequences of *rpoB* gene mutations for *Mtb*-host interactions remain poorly understood. The main aim of the present study was to compare the profiles of cytokines and chemokines of THP-1-derived macrophages in response to an RMR clinical isolate and a susceptible reference *Mtb* strain (H37Rv).

2. Material and methods

2.1. Bacterial strains

H37Rv and RMR *Mtb* strains were used in this study. For phenotypic confirmation, the drug susceptibility test (DST) was performed for isoniazid (0.2 mg/L), rifampin (40 mg/L), streptomycin (4 mg/L), ethambutol (2 mg/L), kanamycin (30 mg/L), ofloxacin (2 mg/L), and capreomycin (40 mg/L), according to the Center for Disease Control and Prevention (CDC) guidelines, using the proportional method. The H37Rv and RMR strains were grown in a liquid medium (7H9 broth), supplemented with 0.2% glycerol, 0.05% Tween 80, and 10% oleic albumin dextrose catalase (OADC). Liquid cultures were grown in tubes (25 mL) at 37 °C for 21–28 days and incubated.

2.2. Whole genome sequencing (WGS)

For this purpose, genomic DNA was extracted from the RMR strain, using the cetyl trimethylammonium bromide (CTAB) method. Libraries were loaded onto the Illumina NextSeq 500 instrument in a paired-end run (2 × 151 bp). The resulting reads were mapped to the *Mtb* H37Rv genome (GenBank ID, NC_000962.3).

2.3. Infection of THP-1

The THP-1 cell line was maintained in the RPMI-1640 medium (Gibco), supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich), 2 mM L-glutamine, streptomycin (100 µg/mL), penicillin (100 U/mL), and nonessential amino acids (1%). Human monocyte THP-1 cell line was grown at 37 °C in 5% CO_2, with passages every three days. The THP-1 cells were plated at a density of 3×10^6 cells and treated with 100 ng/mL of phorbol 12-myristate 13-acetate (PMA; Sigma) to differentiate macrophages after 48 h; they were then left to rest for 24 h without PMA. The confirmation of the monocyte-to-macrophage differentiation was performed as previously described [19]. The macrophages were replaced by fresh RPMI-1640 medium; accordingly, the

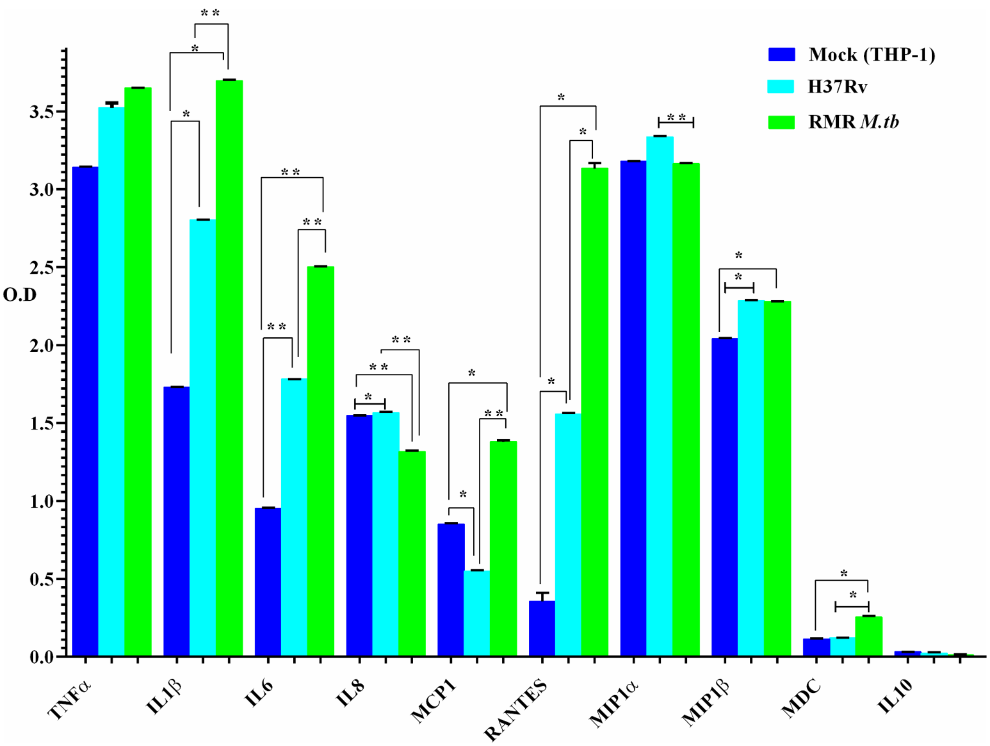

Fig. 2. Production of cytokines and chemokines were measured by ELISA within 24 h after RMR and H37Rv *Mtb* infection. MOI was 10:1 in all experiments ($^{*}p < 0.05$, $^{**}p < 0.001$, and $^{***}p < 0.0001$). The results are shown as the mean + SD of duplicate measurements.

cells were infected with H37Rv and RMR strains. The multiplicity of infection (MOI) was 10:1 (10 *Mtb*: 1 cell) for 24 h in all experiments [20]. The THP-1-derived macrophages were infected with both strains for 2 h, rinsed twice with 1X phosphate-buffered saline (PBS) to eliminate extracellular *Mtb* strains, and incubated at 37 °C for 24 h. To determine the viability of uninfected and infected cells, the trypan blue exclusion test was carried out, based on the manufacturer's instructions (Sigma Aldrich, Germany). Intracellular growth assay (at 2, 4, 24 h and 7 days post infection) and evaluation of intracellular internalization index was performed as previously described [7,21].

2.4. ELISA assay

In the presence or absence of H37Rv and RMR strains, THP-1-derived macrophages were incubated for 24 h. The culture supernatants were analyzed by ELISA assay for cytokine and chemokine products, and their levels were measured following the manufacturer's instructions. All cytokine and chemokine products were used to measure the levels of TNF-α, IL-1β, IL-6, IL-8, monocyte chemotactic protein-1 (MCP-1), RANTES, macrophage inflammatory protein-1α (MIP-1α), MIP-1β and macrophage-derived chemokine (MDC) in a Multi-Analyte ELISArray Kit (Qiagen; Cat. No., MEH-008A). On the other hand, for IL-10 measurements, a Single-Analyte ELISArray Kit (Qiagen; Cat. No., SEH00572A) was used. All assays were performed in biological duplicate.

2.5. RNA extraction, cDNA synthesis, and real-time PCR array

After infection, the supernatants were removed, and total RNA was isolated from macrophages, using RNeasy Mini Kit (Qiagen; Cat. No., 74104), according to the manufacturer's protocols. The RNA quality was determined using NanoDrop. Also, cDNA was synthesized from total RNA of THP-1-derived macrophages, using RT^2 First Strand Kit (Qiagen; Cat. No., 330404), based on the standard method. Then, cDNA was used on Human Cytokines and Chemokines Real-Time RT^2 Profiler PCR Array (Qiagen; Cat. No., PAHS-150Z), using RT^2 SYBR Green qPCR Master Mix (Qiagen; Cat. No., 330529). All assays were performed in biological triplicate. The collected data were entered in the NCBI Gene Expression Omnibus (GEO), with the accession number, GSE115546.

2.6. Statistical analysis

The RT^2 Profiler PCR Array Data Analysis web portal (https://www.qiagen.com/ir/resources/geneglobe/) was used for assessing the threshold cycle (Ct) to calculate changes in gene expression and generate volcano plots and clustergrams. Differences in the mean values of the groups were determined, using student *t* test. Comparisons were made among differentially expressed genes with a fold-change cut-off of two. GraphPad Prism 7 (GraphPad, La Jolla, CA, USA) was used for statistical analyses. *P*-value < 0.05 was considered statistically significant.

3. Results

3.1. DST and WGS findings

The phenotypic DST showed that the studied strain was only resistant to rifampin. Based on the WGS analysis, the only observed mutation was *rpoB* S450L (TCG/tTg) in the RMR strain. There were no other putative resistance markers in this strain; the strain belonged to the lineage 4.8 (mainly T).

3.2. Intracellular internalization and intracellular growth

The RMR and H37Rv strains showed comparable infectivity in THP-1-derived macrophages. In this assay the cells showing phagocytosis were divided into different groups (1–5, 6–10 and greater than 10 bacilli per cell), it was found that percentage of THP-1 cells infected with H37Rv showed no statistically significant difference from RMR strain (Fig. 1a).

Table 1
Fold regulation comparison between control group (uninfected THP-1-derived macrophages), group 1 (RMR *Mtb*), group 2 (H37Rv *Mtb*).

Up-down regulation (comparing to control group)									
Symbol	Group 1(RMR *Mtb*)		Group 2 (H37Rv)		Symbol	Group 1(RMR *Mtb*)		Group 2 (H37Rv)	
	Fold regulation	P Value	Fold regulation	P Value		Fold regulation	P Value	Fold regulation	P Value
ADIPOQ	−3.4903	0.043371	−4.1795	0.03637	IL12A	−3.4903	0.043371	−4.1795	0.03637
BMP2	−1.0401	0.734464	1.5122	0.16216	IL12B	−3.4903	0.043371	−4.1795	0.03637
BMP4	−3.4903	0.043371	−4.1795	0.03637	IL13	−2.4909	0.000734	−1.3044	0.02186
BMP6	2.0326	0.005235	2.9282	0.00458	IL15	−1.0767	0.650134	−4.1795	0.03637
BMP7	−3.4903	0.043371	−4.1795	0.03637	IL16	−3.4903	0.043371	−2.6329	0.06238
C5	−2.0186	0.100768	−2.2346	0.08258	IL17A	−3.4903	0.043371	−4.1795	0.03637
CCL1	1.9543	0.000489	1.7777	0.00024	IL17F	−3.4903	0.043371	−2.6697	0.06115
CCL11	−3.4903	0.043371	−4.1795	0.03637	IL18	−1.3195	0.000022	−2.4172	0.00257
CCL13	−3.4903	0.043371	−4.1795	0.03637	IL1A	1.5369	0.000076	1.3134	1.4E-05
CCL17	−3.4903	0.043371	−4.1795	0.03637	IL1B	1.6625	0.000002	1.7171	0
CCL18	−2.6945	0.060246	−3.5064	0.04319	IL1RN	1.8532	0	1.1487	0.00348
CCL19	−3.4903	0.043371	−4.1795	0.03637	IL2	−3.4903	0.043371	−4.1795	0.03637
CCL2	1.1277	0.000259	1.3947	0.00018	IL21	−3.4903	0.043371	−4.1795	0.03637
CCL20	1.2658	0.000007	−16.8343	0.56303	IL22	−3.4903	0.043371	−4.1795	0.03637
CCL21	−3.4903	0.043371	−4.1795	0.03637	IL23A	1.8965	0.000008	2.2974	0
CCL22	1.815	0	1.7695	1E-06	IL24	1.2226	0.000967	−1.0187	0.11612
CCL24	−3.4903	0.043371	−4.1795	0.03637	IL27	−3.4903	0.043371	−3.302	0.04621
CCL3	1.0329	0.512256	1.154	9E-06	IL3	−3.4903	0.043371	−4.1795	0.03637
CCL5	1.7942	0.000011	1.1355	0.00184	IL4	−3.4903	0.043371	−4.1795	0.03637
CCL7	−1.4208	0.000056	−1.0329	0.11612	IL5	−3.4903	0.043371	−4.1795	0.03637
CCL8	−3.3792	0.044993	−4.1795	0.03637	IL6	1.2397	0.000157	−1.0943	0.00017
CD40LG	−5.1694	0	−1.9816	0	IL7	−3.4903	0.043371	−4.1795	0.03637
CNTF	−2.6329	0.062382	−1.3348	0.30848	CXCL8	1.0521	0.037826	−1.1487	0.00213
CSF1	2.0801	0	2.1685	0.00033	IL9	−3.4903	0.043371	−4.1795	0.03637
CSF2	−1.2483	0.386311	1.0943	0.89769	LIF	5.0397	0.000059	5.4264	0
CSF3	1.0281	0.119813	−1.4607	3.9E-05	LTA	−3.4903	0.043371	−2.5787	0.06438
CX3CL1	−3.302	0	−3.3326	0	LTB	−3.4903	0.043371	−4.1795	0.03637
CXCL1	−1.0693	0.042334	−1.1728	0.00025	MIF	1.5087	0.000032	1.0644	0.0122
CXCL10	1.4709	0.194709	−2.0994	0.09274	MSTN	−3.4903	0.043371	−3.302	0.04621
CXCL11	−3.4903	0.043371	−4.1795	0.03637	NODAL	−3.4903	0.043371	−4.1795	0.03637
CXCL12	−2.4967	0.067796	−2.1386	0.08942	OSM	−3.4903	0.043371	−3.0035	0.05184
CXCL13	−3.4903	0.043371	−4.1795	0.03637	PPBP	−1.1728	0.000246	−1.4241	3.6E-05
CXCL16	−1.9097	0	−1.4142	8E-06	SPP1	1.341	0.000011	−1.2397	6.8E-05
CXCL2	1.3013	0.000005	1.1251	0.00605	TGFB2	−3.4903	0.043371	−2.2089	0.08417
CXCL5	1.6434	0.000005	1.6021	1E-06	THPO	−3.4903	0.043371	−4.1795	0.03637
CXCL9	−3.4903	0.043371	−3.7235	0.04056	TNF	1.0093	0.462113	1.4175	0.00023
FASLG	−3.4903	0.043371	−4.1795	0.03637	TNFRSF11B	−3.3636	0.045218	−3.0809	0.05024
GPI	1.2983	0.000248	1.6896	0	TNFSF10	−3.4903	0.043371	−1.7053	0.14918
IFNA2	−3.4903	0.043371	−4.1795	0.03637	TNFSF11	−3.4903	0.043371	−4.1795	0.03637
IFNG	−3.4903	0.043371	−4.1795	0.03637	TNFSF13B	−3.4903	0.043371	−4.1795	0.03637
IL10	−1.8025	0.111661	−1.1783	0.45576	VEGFA	1.4142	0.00003	1.181	0.00039
IL11	−3.4903	0.043371	−4.1795	0.03637	XCL1	−3.4903	0.043371	−4.1795	0.03637

The intracellular growth of both studied strains was evaluated by CFU counts at 2, 4, 24 h and 7 days post infection. No significant differences were observed in the intracellular CFUs at 2 and 4 h post infection, but RMR strain showing significantly higher intracellular bacillary growth compared to H37Rv after 1 and 7 days (Fig. 1b). Macrophages infected by two strains showed similar viability, as indicated by trypan blue exclusion assay indicating that cytotoxicity may not account for the observed differences of CFUs.

3.3. Cytokine and chemokine secretion patterns

Cytokine and chemokine secretion patterns were studied in both infected cells and compared with mock cells by ELISA assay. THP-1-derived macrophages infected with RMR *Mtb* expressed higher levels of cytokine and chemokine products in the supernatant for IL-1β, IL-6, MCP-1, RANTES, and MDC, compared to both H37Rv-infected and control cells (Fig. 2). On the other hand, a significant reduction was observed in IL-8 secretion in response to RMR, compared to H37Rv-infected and control cells (Fig. 2).

3.4. Upregulated genes in response to RMR and H37Rv Mtb strains

The gene expression of cytokines and chemokines was determined by qRT-PCR assay (Table 1). The minority of genes, which were expressed in response to both *Mtb* infections, were upregulated, compared to the control group. Upregulation of *CSF-1*, *LIF*, and *BMP-6* genes was identified in both infected cells. The *IL23A* gene was only significantly upregulated in response to H37Rv infection. The upregulated genes in infected THP-1-derived macrophages were more expressed by the H37Rv *Mtb* strain, compared to the RMR strain.

3.5. Downregulated genes in response to RMR and/or H37Rv Mtb strains

The majority of genes, which were differentially expressed in response to both virulent *Mtb* strain infections, were downregulated, compared to the control group (IL-12 family, IFN-γ, IFN-α, TGF-β, and some chemokines). Among downregulated genes, *CXCL12*, *IL-16*, *1L-17F*, *1L-27*, *LTA*, *MSTN*, *OSM*, *TGFβ-2*, and *TNFRSF11B* genes were downregulated more in THP-1-derived macrophages with RMR infection, compared to those infected with the H37Rv strain. However, other downregulated genes were more significantly expressed in THP-1-derived macrophages infected with the H37Rv strain, compared to those

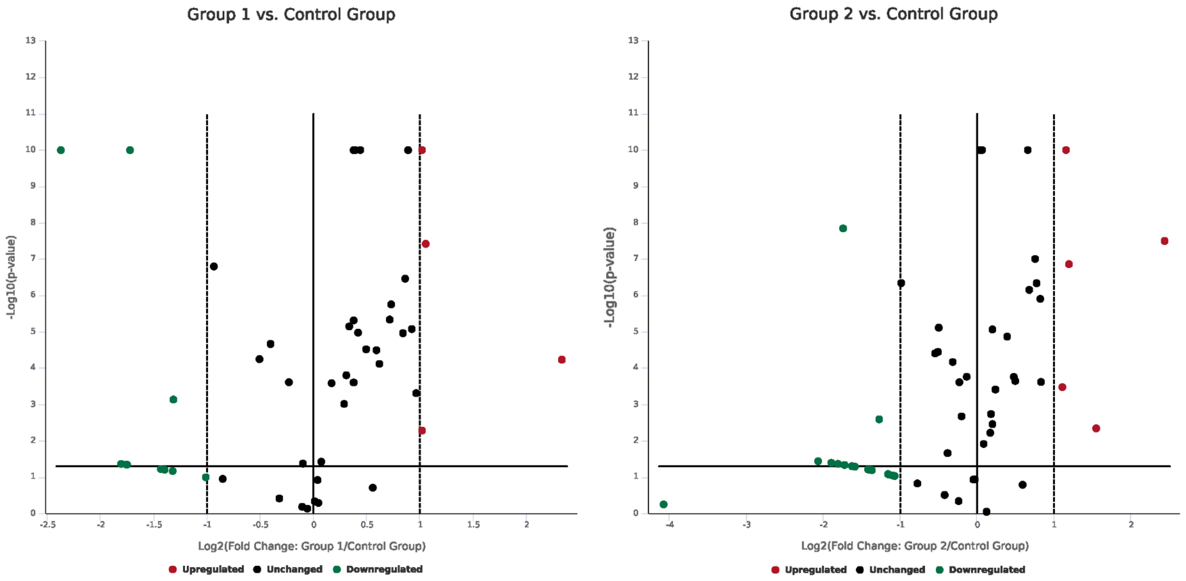

Fig. 3. Differential expression analysis results for 84 cytokines and chemokines gene stimulated with RMR (Group 1) and H37Rv (Group 2) *Mtb* strains. This plot displays the statistical significance versus fold-change on the y- and x-axes, respectively. Upregulated, unchanged and downregulated expressed genes are plotted in red, black and green, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

infected with RMR.

The results of gene analysis, confirmed by qRT-PCR array, showed that *CCL20*, *CXCL10*, *IL-15*, and *IL-18* genes were downregulated by the H37Rv *Mtb* strain, while they remained unchanged in exposure to the RMR strain. On the other hand, several genes, including *IL-13*, *CD40LG*, *CNTF*, and *TNFSF10*, were downregulated by RMR, although they remained unchanged in response to the H37Rv strain. All gene expression profiles are depicted in Figs. 3 and 4.

4. Discussion

The effect of *Mtb* drug resistance on the host–bacterium interactions remains largely unexplored. Therefore, it seems important to evaluate the impact of individual drug-resistant mutations in *Mtb* on the host immunological responses. We performed this comparative study in order to evaluate the cytokine and chemokine expression profiles of THP-1-derived macrophages in exposure to an RMR clinical isolate and H37Rv *Mtb* strain. The RMR strain had two important characteristics. First, based on the WGS findings, it only contained the *rpoB* S450L mutation in RRDR, while there were no putative compensatory mutations in *rpoA/B/C* or other resistance markers, making it a suitable model for studying the effects of single-drug resistant mutations on the host-pathogen interactions. Second, WGS data revealed that the RMR strain belonged to lineage 4 similar to the H37Rv strain. Therefore, the impact of *Mtb* genotype, as one of the important and effective factors, reduced significantly. One of the important factors in this regard was internalization of bacteria, which no significant differences were observed between two strains in our study. Therefore, difference in observed cellular response cannot be affected by initial bacterial burden.

Production of soluble mediators, such as cytokines and chemokines from macrophages, is one of the most important immune responses to this intracellular bacterium [22–24]. TNF-α family is crucial for initiating an effective immune response against TB, which triggers bacteriostatic macrophage functions [25,26]. Although in the current study, there was no significant dysregulation of TNF-α at mRNA and translational levels, other members of the TNF superfamily, such as *TNFSF-11*, *TNFSF-11B*, and *TNFSF-13B*, were downregulated by the *Mtb* strains. Similarly, Howard et al. showed that mRNA and protein levels of TNF-α were comparable in drug susceptible and drug resistant *Mtb*-infected macrophages [27]. These observations are in contrast with the findings reported by Wong et al., who showed that the level of TNF-α, secreted after 24 h of infection, was lower in the presence of a virulent strain, compared to a less virulent strain [28].

Inflammatory and immune response genes of IFNs are involved in early cellular responses, induced by macrophage models. A recent study demonstrated that the induction mechanisms of *IFN-I* genes may differ in *Mtb* strains; therefore, production of IFN-I is related to consequent incidents during an *Mtb* infection [29]. A recent study also showed that IFN-I exhibited both adversary and protective activities against TB. Negative and positive regulations were induced at low and high levels of type-I IFN signaling, and protective functions and hostile effects of IFN-I on *Mtb* were described [30]. Our findings showed that *IFN-γ* was downregulated in response to both strains, which might dampen the immunomodulatory role of IFN-γ against *Mtb* strains [31]. Moreover, at the translational level, the RMR strain upregulated the level of IFN-β2 (IL-6) more than the H37Rv strain (Fig. 2). Dutta et al. revealed that IL-6 inhibits IFN-γ-induced autophagy in *Mtb*-infected macrophages [32]. Howard et al. recently proved that *Mtb* with *rpoB* (H445Y) mutation in Il1r1−/− macrophages showed increased IFN-β signaling [27]. Our findings also clearly indicate that both *Mtb* strains, especially the RMR strain, can upregulate IL-6 production by macrophages to regulate host immunity.

The IL-12 cytokine family is stimulated after activation of other cytokines, organizing the Th1 response and contributing to immune responses during *Mtb* infections [33]. Several studies have demonstrated that the relationship between IL-23 and IL-17 pathways may play a critical role in controlling *Mtb* infections [34,35]. A recent study highlighted that a balance in IL-23 level is essential for both high and low expression of inflammatory cytokines, which can lead to pathology and loss of immunity, respectively [36]. We detected different activities of IL-12 cytokine family. *IL-12A*, *IL-12B*, *1L-27*, *IL-17A*, and *IL-17F* genes were downregulated in response to RMR and H37Rv strains. On the other hand, the *IL-23α* gene was more significantly activated by the H37Rv strain in human macrophages, compared to the RMR strain. It seems that inducing or blocking the IL-12 family in mycobacterial diseases is not attributed to the resistance profile of *Mtb*.

The IL-1 cytokine family, including IL-1α, IL-1β, and IL-18, has diverse immunological activities in response to inflammation, immune response regulation, and optimal control of *Mtb* infection. IL-1α is an effective cytokine, which is involved in the induction of host responses

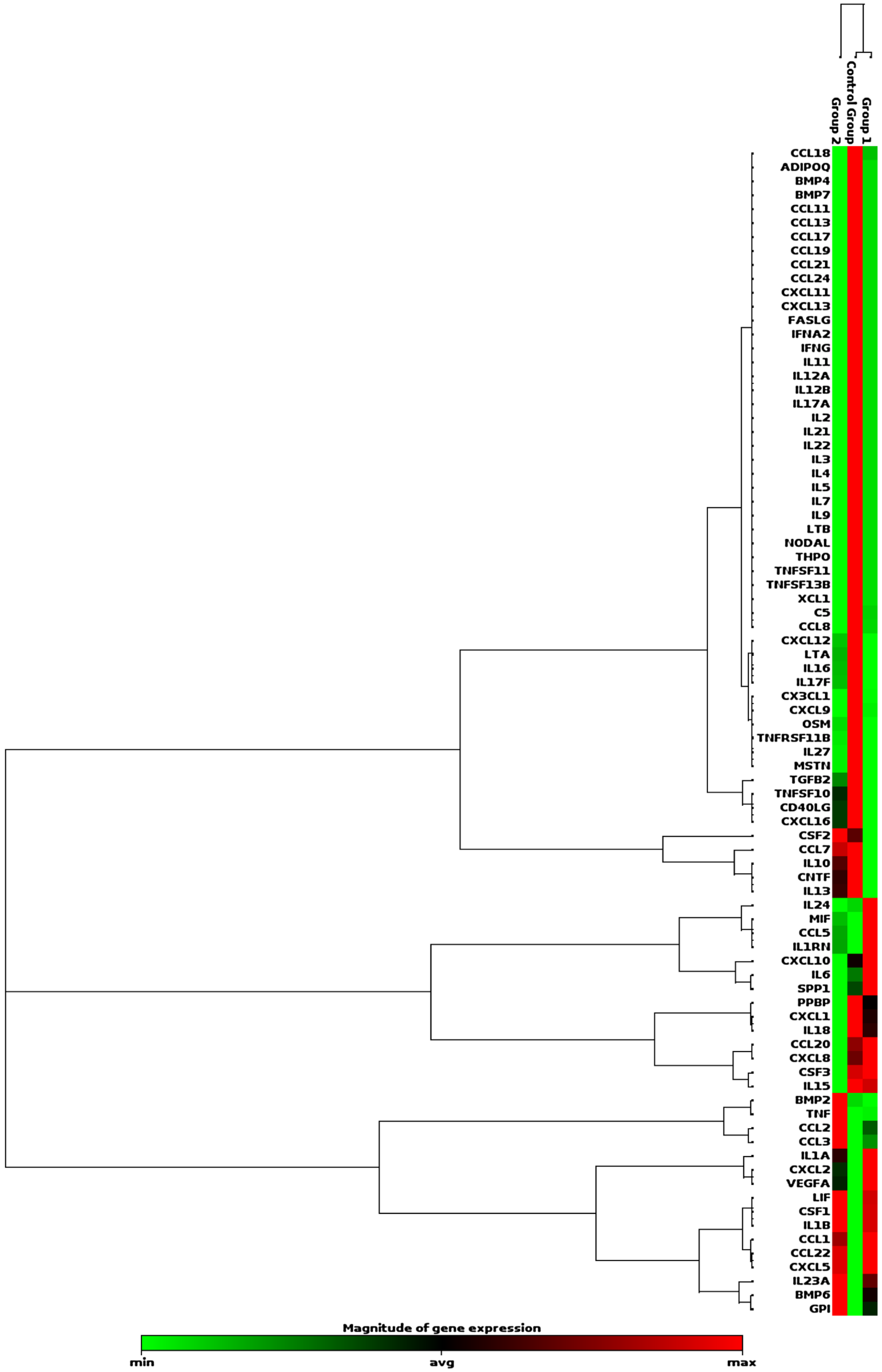

Fig. 4. Clustergram analysis, the mRNA expression profile of cytokines and chemokines gene from the THP-1-derived macrophages infected by RMR and H37Rv *Mtb* strains are shown as a heatmap graph of gene expression data. The 84 genes were clustered conforming to their expression patterns. This clustergram performs nonsupervised hierarchical clustering of the entire dataset to display a heat map with dendrograms indicating co-regulated genes.

to inflammation and cell damage and plays a critical role in host protection against *Mtb* [37,38]. A recent study demonstrated that IL-1β is directly associated with IL-1α, acting as a proxy for other proinflammatory cytokines [39]. According to our findings, *IL-1α*, *IL-1β*, and *IL-1RN* (inhibitor of IL-1 activity) remained unchanged by the *Mtb* strains. We observed that IL-1β expression increased significantly in response to both strains, especially the RMR *Mtb* strain. However, this phenomenon cannot be attributed to the differential IL-1β mRNA transcription. This finding is in agreement with a study by Krishnan, who revealed that different clinical strains of *Mtb* use a specific pathway to stimulate IL-1β secretion from macrophages [40]. IL-18 is also a cytokine produced by macrophages. It is significant for IFN-γ production in response to *Mtb* and is associated with both IL-12 and IL-27. In addition, Robinson et al. explored the contribution of IL-18, TNF-α, and IFN-γ cytokines, which play a significant role in controlling the growth of *Mtb* in human macrophages [41]. In our study, *IL-18* was downregulated and unchanged in response to H37Rv and RMR strains, respectively. On the other hand, *IL-12*, *IL-27*, and *IFN-γ* showed similar expression patterns (downregulated by RMR and H37Rv strains). However, further studies are required to understand the influence of IL-1 family and its friendly interactions on limiting the growth of *Mtb* in primary human macrophages.

IL-8 (CXCL8) is another important cytokine, examined in our study. Although there were no significant changes at the transcriptional level in response to the strains, we observed a significant reduction in IL-8 in the supernatant of THP-1-derived macrophages infected by RMR *Mtb*; therefore, reduction of this cytokine can be the mechanism of RMR bacteria for immune evasion. Sia et al. showed that interactions between CD40 and CD40L are critical to the production of IL-17 during an *Mtb* infection [42]. In our study, significant reduction of *CD40LG* expression by the RMR strain could have survival advantages for this drug-resistant bacterium. Additionally, *CNTF*, *IL-13*, and *TNFSF10* genes were downregulated in response to RMR, but remained unchanged in response to the H37Rv strain; this may indicate the recruitment and control of macrophage early responses by this resistant strain.

Chemokines play a critical role in initial responses to macrophages during *Mtb* infections. Under particular conditions, chemokines can play inconsistent roles in improving the immune system, causing inflammation and lung damage [43,44]. Several studies have shown that macrophages produce chemokines, such as CCL2 (MCP-1), CCL3 (MIP-1α), CCL4 (MIP-1β), and CCL5 (RANTES) in response to virulent *Mtb* [45,46]. Saukkonen et al. also found that MIP-1β and RANTES inhibited the intracellular increase in *Mtb* [47]. In our study, both *Mtb* strains downregulated most chemokines, compared to the control cells. *CCL1*, *MCP-1*, *MIP-1-α*, *RANTES*, *CCL7*, and *CCL22* genes remained unchanged, while *CCL8*, *CCL11*, *CCL13*, *CCL17*, *CCL18*, *CCL19*, *CCL21*, and *CCL24* genes were downregulated at the mRNA level. However, at the translational level, MCP-1 and RANTES were significantly upregulated by the RMR strain.

One of our interesting results was that RMR strain showing significantly higher intracellular bacillary growth compared to H37Rv strain specially after 1 and 7 days. The most important explanation for this phenomenon is that biosynthesis of cell wall lipids, including phthiocerol dimycocerosate (PDIM) is upregulated in *rpoB* mutants which has been proved by others [48,49]. Several roles for PDIM function in bacterial pathogenesis have been proposed, including resistance to killing by nitric oxide and more importantly masking of microbial-associated molecular patterns [50]. The higher intracellular bacillary growth observed with RMR strain may correlate with its ability to spread in communities. The results of the current study are in concordance with the epidemiological studies which show that *Mtb* strains carrying *rpoB* S450L are able to outcompete other rifampicin resistance mutations due to its low fitness cost. This will lead to dominancy of these strains carrying the *rpoB* S450L mutation in the population [51,52].

Collectively, although the macrophages immune responses induced in response to infection with drug-resistant *Mtb* (*e.g.* RMR strain) may not be substantially different from drug susceptible *Mtb* (*e.g.* H37Rv strain), but the severity of the responses was different in some cases specially IL-6, IL-8, *CNTF*, *IL-13*, *TNFSF10* and *CD40LG* in response to RMR and H37Rv strains. In addition, RMR strain showing significantly higher intracellular bacillary growth compared to H37Rv strain. These results initially demonstrate that the *Mtb* carrying the *rpoB*-S450L can modulate macrophage responses to mediate bacterial survival.

5. Conclusions

The current study shed more light on the fitness cost of RMR strain with no putative compensatory mutations. Comparison of mono-resistant and susceptible *Mtb* strains is important not only for studying the effect of specific mutation on downstream host pathogen interactions but also can provide valuable data at population level. In addition, how particular drug resistance mutations (*e.g.*, *rpoB* S450L) can alter host–pathogen interactions, and how this might impact the development of new therapeutics can be interesting subjects for further investigations [53].

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Conflict of interest

The authors declare that they have no conflicting interests.

References

[1] Organization WHO: Global tuberculosis report 2017, 2017.
[2] J.B. Torrelles, L.S. Schlesinger, Integrating lung physiology, immunology, and tuberculosis, Trends Microbiol. 25 (8) (2017) 688–697.
[3] L. Lavalett, H. Rodriguez, H. Ortega, W. Sadee, L.S. Schlesinger, L.F. Barrera, Alveolar macrophages from tuberculosis patients display an altered inflammatory gene expression profile, Tuberculosis 107 (2017) 156–167.
[4] D. Portevin, S. Gagneux, I. Comas, D. Young, Human macrophage responses to clinical isolates from the Mycobacterium tuberculosis complex discriminate between ancient and modern lineages, PLoS Pathog 7 (3) (2011) e1001307.
[5] S. Chatterjee, The lung immune niche in tuberculosis: insights from studies on human alveolar macrophages, Curr. Trop. Med. Rep. 2 (2) (2015) 49–53.
[6] E. Guirado, L.S. Schlesinger, G. Kaplan, Macrophages in tuberculosis: friend or foe, Semin. Immunopathol. 35 (5) (2013) 563–583.
[7] P. Chakraborty, S. Kulkarni, R. Rajan, K. Sainis, Drug resistant clinical isolates of Mycobacterium tuberculosis from different genotypes exhibit differential host responses in THP-1 cells, PloS One 8 (5) (2013) e62966.
[8] C. Manca, M.B. Reed, S. Freeman, B. Mathema, B. Kreiswirth, C.E. Barry, G. Kaplan, Differential monocyte activation underlies strain-specific Mycobacterium tuberculosis pathogenesis, Infect. Immun. 72 (9) (2004) 5511–5514.
[9] W. Chanput, J.J. Mes, H.J. Wichers, THP-1 cell line: an in vitro cell model for immune modulation approach, Int. Immunopharmacol. 23 (1) (2014) 37–45.
[10] X. Bai, S.-H. Kim, T. Azam, M.T. McGibney, H. Huang, C.A. Dinarello, E.D. Chan, IL-32 is a host protective cytokine against Mycobacterium tuberculosis in differentiated THP-1 human macrophages, J. Immunol. 184 (7) (2010) 3830–3840.
[11] A.Z. Castro, B.M. Diaz-Bardalez, E.C. Oliveira, R.C. Garcia, J.B. Afiune, I.A. Paschoal, L.M. Santos, Abnormal production of transforming growth factor β and interferon γ by peripheral blood cells of patients with multidrug-resistant pulmonary tuberculosis in Brazil, J. Infect. 51 (4) (2005) 318–324.
[12] Y.E. Wu, W.G. Peng, Y.M. Cai, G.Z. Zheng, G.L. Zheng, J.H. Lin, S.W. Zhang, K. Li, Decrease in CD4+ CD25+ FoxP3+ Treg cells after pulmonary resection in the treatment of cavity multidrug-resistant tuberculosis, Int. J. Infect. Dis. 14 (9) (2010) e815–e822.
[13] Y. Pang, J. Lu, Y. Wang, Y. Song, S. Wang, Y. Zhao, Study of the rifampin monoresistance mechanism in Mycobacterium tuberculosis, Antimicrob. Agents Chemother. 57 (2) (2013) 893–900.
[14] J.-I. Sekiguchi, T. Miyoshi-Akiyama, E. Augustynowicz-Kopeć, Z. Zwolska, F. Kirikae, E. Toyota, I. Kobayashi, K. Morita, K. Kudo, S. Kato, Detection of multidrug resistance in Mycobacterium tuberculosis, J. Clin. Microbiol. 45 (1) (2007) 179–192.
[15] T. Song, Y. Park, I.C. Shamputa, S. Seo, S.Y. Lee, H.S. Jeon, H. Choi, M. Lee, R.J. Glynne, S.W. Barnes, Fitness costs of rifampicin resistance in Mycobacterium tuberculosis are amplified under conditions of nutrient starvation and compensated by mutation in the β′ subunit of RNA polymerase, Mol. Microbiol. 91 (6) (2014) 1106–1119.
[16] Y.M. Coovadia, S. Mahomed, M. Pillay, L. Werner, K. Mlisana, Rifampicin monoresistance in Mycobacterium tuberculosis in KwaZulu-Natal, South Africa: a significant phenomenon in a high prevalence TB-HIV region, PLoS One 8 (11) (2013) e77712.
[17] V. Meyssonnier, T. Van Bui, N. Veziris, V. Jarlier, J. Robert, Rifampicin mono-resistant tuberculosis in France: a 2005–2010 retrospective cohort analysis, BMC Infect. Dis. 14 (1) (2014) 18.
[18] A. Koch, V. Mizrahi, D.F. Warner, The impact of drug resistance on Mycobacterium

tuberculosis physiology: what can we learn from rifampicin? Emerg. Microbes. Infect. 3 (3) (2014) e17.

[19] M. Genin, F. Clement, A. Fattaccioli, M. Raes, C. Michiels, M1 and M2 macrophages derived from THP-1 cells differentially modulate the response of cancer cells to etoposide, BMC Cancer 15 (1) (2015) 577.

[20] X. Wang, Y. Wu, J. Jiao, Q. Huang, Mycobacterium tuberculosis infection induces IL-10 gene expression by disturbing histone deacetylase 6 and histonedeacetylase 11 equilibrium in macrophages, Tuberculosis 108 (2018) 118–123.

[21] A. Romagnoli, E. Petruccioli, I. Palucci, S. Camassa, E. Carata, L. Petrone, S. Mariano, M. Sali, L. Dini, E. Girardi, Clinical isolates of the modern Mycobacterium tuberculosis lineage 4 evade host defense in human macrophages through eluding IL-1β-induced autophagy, Cell Death Dis. 9 (6) (2018) 624.

[22] E. Vergadi, E. Ieronymaki, K. Lyroni, K. Vaporidi, C. Tsatsanis, Akt signaling pathway in macrophage activation and M1/M2 polarization, J. Immunol. 198 (3) (2017) 1006–1014.

[23] G. Prosser, J. Brandenburg, N. Reiling, C.E. Barry III, R.J. Wilkinson, K.A. Wilkinson, The bacillary and macrophage response to hypoxia in tuberculosis and the consequences for T cell antigen recognition, Microbes Infect. 19 (3) (2017) 177–192.

[24] C. Cambier, S. Falkow, L. Ramakrishnan, Host evasion and exploitation schemes of Mycobacterium tuberculosis, Cell 159 (7) (2014) 1497–1509.

[25] E. Iona, M. Pardini, M.C. Gagliardi, M. Colone, A.R. Stringaro, R. Teloni, L. Brunori, R. Nisini, L. Fattorini, F. Giannoni, Infection of human THP-1 cells with dormant Mycobacterium tuberculosis, Microbes Infect. 14 (11) (2012) 959–967.

[26] N. Parameswaran, S. Patial, Tumor necrosis factor-α signaling in macrophages, Crit. Rev. Eukaryot Gene Expr. 20 (2) (2010).

[27] N.C. Howard, N.D. Marin, M. Ahmed, B.A. Rosa, J. Martin, M. Bambouskova, A. Sergushichev, E. Loginicheva, N. Kurepina, J. Rangel-Moreno, Mycobacterium tuberculosis carrying a rifampicin drug resistance mutation reprograms macrophage metabolism through cell wall lipid changes, Nat. Microbiol. 3 (10) (2018) 1099–1108.

[28] K. Wong, W. Leong, H. Law, K. Ip, J. Lam, K. Yuen, P. Ho, W. Tse, X. Weng, W. Zhang, Molecular characterization of clinical isolates of Mycobacterium tuberculosis and their association with phenotypic virulence in human macrophages, Clin. Vaccine Immunol. 14 (10) (2007) 1279–1284.

[29] N. Reiling, S. Homolka, T.A. Kohl, C. Steinhäuser, K. Kolbe, S. Schütze, J. Brandenburg, Shaping the niche in macrophages: genetic diversity of the M. tuberculosis complex and its consequences for the infected host, Int. J. Med. Microbiol. (2017).

[30] L. Moreira-Teixeira, K. Mayer-Barber, A. Sher, A. O'Garra, Type I interferons in tuberculosis: Foe and occasionally friend, J. Exp. Med. 215 (5) (2018) 1273–1285.

[31] S. Pahari, N. Khan, M. Aqdas, S. Negi, J. Kaur, J.N. Agrewala, Infergen stimulated macrophages restrict Mycobacterium tuberculosis growth by autophagy and release of nitric oxide, Sci. Rep. 6 (2016) 39492.

[32] R.K. Dutta, M. Kathania, M. Raje, S. Majumdar, IL-6 inhibits IFN-γ induced autophagy in Mycobacterium tuberculosis H37Rv infected macrophages, Int. J. Biochem. Cell Biol. 44 (6) (2012) 942–954.

[33] S.P. Hickman, J. Chan, P. Salgame, Mycobacterium tuberculosis induces differential cytokine production from dendritic cells and macrophages with divergent effects on naive T cell polarization, J. Immunol. 168 (9) (2002) 4636–4642.

[34] S.A. Khader, G.K. Bell, J.E. Pearl, J.J. Fountain, J. Rangel-Moreno, G.E. Cilley, F. Shen, S.M. Eaton, S.L. Gaffen, S.L. Swain, IL-23 and IL-17 in the establishment of protective pulmonary CD4+ T cell responses after vaccination and during Mycobacterium tuberculosis challenge, Nat. Immunol. 8 (4) (2007) 369.

[35] S.A. Khader, S.L. Gaffen, J.K. Kolls, Th17 cells at the crossroads of innate and adaptive immunity against infectious diseases at the mucosa, Mucosal. Immunol. 2 (5) (2009) 403.

[36] S.A. Khader, L. Guglani, J. Rangel-Moreno, R. Gopal, B.A.F. Junecko, J.J. Fountain, C. Martino, J.E. Pearl, M. Tighe, Y.-Y. Lin, IL-23 is required for long-term control of Mycobacterium tuberculosis and B cell follicle formation in the infected lung, J. Immunol. 187 (10) (2011) 5402–5407.

[37] N.C. Di Paolo, S. Shafiani, T. Day, T. Papayannoupoulou, D.W. Russell, Y. Iwakura, D. Sherman, K. Urdahl, D.M. Shayakhmetov, Interdependence between interleukin-1 and tumor necrosis factor controls TNF-dependent effector functions during Mycobacterium tuberculosis infection, Immunity 43 (6) (2015) 1125.

[38] A.M. Cooper, K.D. Mayer-Barber, A. Sher, Role of innate cytokines in mycobacterial infection, Mucosal Immunol. 4 (3) (2011) 252.

[39] A. Fettelschoss, M. Kistowska, S. LeibundGut-Landmann, H.-D. Beer, P. Johansen, G. Senti, E. Contassot, M.F. Bachmann, L.E. French, A. Oxenius, Inflammasome activation and IL-1β target IL-1α for secretion as opposed to surface expression, Proc. Natl. Acad. Sci. USA 108 (44) (2011) 18055–18060.

[40] N. Krishnan, B.D. Robertson, G. Thwaites, Pathways of IL-1β secretion by macrophages infected with clinical Mycobacterium tuberculosis strains, Tuberculosis 93 (5) (2013) 538–547.

[41] C. Robinson, G. Nau, IL-18, TNF-α, and IFN-γ are involved in a network of cytokine interactions that promote control of Mycobacterium tuberculosis by human macrophages, J. Immunol. 184 (2010) 37–52.

[42] J.K. Sia, E. Bizzell, R. Madan-Lala, J. Rengarajan, Engaging the CD40-CD40L pathway augments T-helper cell responses and improves control of Mycobacterium tuberculosis infection, PLoS Pathog. 13 (8) (2017) e1006530.

[43] R. Domingo-Gonzalez, O. Prince, A. Cooper, S. Khader, Cytokines and chemokines in Mycobacterium tuberculosis infection, Microbiol. Spectr. 4 (5) (2016).

[44] L. Monin, S.A. Khader, Chemokines in tuberculosis: the good, the bad and the ugly, Semin. Immunol. 26 (6) (2014) 552–558.

[45] L. Qiu, D. Huang, C.Y. Chen, R. Wang, L. Shen, Y. Shen, R. Hunt, J. Estep, B.F. Haynes, W.R. Jacobs Jr, Severe tuberculosis induces unbalanced up-regulation of gene networks and overexpression of IL-22, MIP-1α, CCL27, IP-10, CCR4, CCR5, CXCR3, PD1, PDL2, IL-3, IFN-β, TIM1, and TLR2 but low antigen-specific cellular responses, J. Infect. Dis. 198 (10) (2008) 1514–1519.

[46] M.I. Sadek, E. Sada, Z. Toossi, S.K. Schwander, E.A. Rich, Chemokines induced by infection of mononuclear phagocytes with mycobacteria and present in lung alveoli during active pulmonary tuberculosis, Am. J. Respir. Cell Mol. Biol. 19 (3) (1998) 513–521.

[47] J.J. Saukkonen, B. Bazydlo, M. Thomas, R.M. Strieter, J. Keane, H. Kornfeld, β-Chemokines are induced by Mycobacterium tuberculosis and inhibit its growth, Infect. Immun. 70 (4) (2002) 1684–1693.

[48] G.P. Bisson, C. Mehaffy, C. Broeckling, J. Prenni, D. Rifat, D.S. Lun, M. Burgos, D. Weissman, P.C. Karakousis, K. Dobos, Upregulation of the phthiocerol dimycocerosate biosynthetic pathway by rifampicin-resistant, rpoB-mutant Mycobacterium tuberculosis, J. Bacteriol. 194 (23) (2012) 6441–6452.

[49] N. Lahiri, R.R. Shah, E. Layre, D. Young, C. Ford, M.B. Murray, S.M. Fortune, D.B. Moody, Rifampin resistance mutations are associated with broad chemical remodeling of Mycobacterium tuberculosis, J. Biol. Chem. 291 (27) (2016) 14248–14256.

[50] C. Cambier, K.K. Takaki, R.P. Larson, R.E. Hernandez, D.M. Tobin, K.B. Urdahl, C.L. Cosma, L. Ramakrishnan, Mycobacteria manipulate macrophage recruitment through coordinated use of membrane lipids, Nature 505 (7482) (2014) 218–222.

[51] S.M. Gygli, S. Borrell, A. Trauner, S. Gagneux, Antimicrobial resistance in Mycobacterium tuberculosis: mechanistic and evolutionary perspectives, FEMS Microbiol. Rev. 41 (3) (2017) 354–373.

[52] F. Vaziri, T.A. Kohl, H. Ghajavand, M. Kargarpour Kamakoli, M. Merker, S. Hadifar, S. Khanipour, A. Fateh, M. Masoumi, S.D. Siadat, S. Niemann, The genetic diversity of multi and extensively drug resistant Mycobacterium tuberculosis in the capital of Iran revealed by whole genome sequencing, J. Clin. Microbiol. 57 (2019) e01477–e1518.

[53] Y.-X. Chen, B. Javid, More than merely drug resistance, Nat. Microbiol. 3 (10) (2018) 1078–1079.