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Comparison of human monocyte derived macrophages and THP1-like macrophages as *in vitro* models for *M. tuberculosis* infection

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ABSTRACT

Macrophages are the preferential cell types to study various aspects of mycobacterial infection. Commonly used infection models for *in-vitro* studies are primary macrophages such as human monocyte derived macrophages (hMDMs) and macrophage like cell lines (THP-1). It is not clear if commercially available THP-1 cells can be used as hMDMs alternative for *in-vitro M.tb* infection experiments. We conducted a detailed investigation of the hMDM and THP-1 response to mycobacterial infection on a comparative basis and assess the most crucial aspects of infection which are most commonly studied. We assessed mycobacterial uptake and intracellular growth over time of a pathogenic drug-resistant and drug-susceptible *M.tb* strains (R179 and H37Rv) through colony forming units (CFUs). Both strains depicted similar uptake and intracellular growth in hMDMs and THP-1 macrophages over time (R179, $p = 0.954$) (H37Rv, $p = 0.922$). Cytotoxicity assays revealed a consistent viability up to day 16 post-infection across the strains in both THP-1 and hMDMs (R179, $p = 0.271$) (H37Rv, $p = 0.068$). Interestingly, both cell lines showed similar mycobacterial uptake and cellular viability in both susceptible as well as resistant *M.tb* strains. Cytokine/chemokine mRNA analysis through qPCR found no difference between cell types. Further, cytokine secretion measured through Luminex revealed no difference across the strains. Also, cytokine secretion analysis showed no difference in both cell lines across strains. In conclusion, our study shows that THP-1 and hMDMs bacterial uptake, viability and host response to drug-susceptible and drug-resistant mycobacterial infections are similar. Therefore, present study demonstrate that THP-1 cells are suitable substitutes for hMDMs for *in-vitro M.tb* infection experiments.

1. Introduction

Macrophages are the first line of defense against any invading pathogen [1] and play a key role in the elimination of mycobacteria. In susceptible individuals, macrophages provide a niche for its replication [2], and are therefore studied in-depth in an attempt to unravel the events at the host-pathogen interface early post-infection. Macrophages as models for mycobacterial infection studies are thus central to the advancement of the current understanding of host-pathogen interactions and are widely used amongst researchers in the field.

There are various macrophage models in use which include primary macrophages as well as macrophage cell lines to study *M. tuberculosis* (*M.tb*) infection. Primary macrophages include human monocyte derived macrophages (hMDMs) and mouse bone marrow-derived macrophages (BMDMs). There are a number of cell lines depicting macrophage models such as the human THP-1 and U937 and the murine RAW264.7 and J774 cell lines [3]. The advantage of using macrophages from *Mus musculus* is that there is substantially lower variability between mice when compared to the variability observed in *Homo sapiens* [4]. *Ex vivo* human macrophages as models for infection are however preferred, with variability between individuals being controlled for best by increasing sample size. hMDMs are isolated from the natural host and are comparatively easy to differentiate *in vitro* from human blood monocytes. However, to draw blood from humans, ethical permission is required which is often a lengthy process. In contrast, cell lines are homogenous, easy to proliferate and easier to maintain in the laboratory. There is the disadvantage that cell lines derived from transformed or immortalized cells have a tendency to be genetically unstable and may exhibit uncharacteristic/aberrant signaling mechanisms [5].

As mentioned previously, hMDMs are considered to be the first line of defense against mycobacterial infection due to their extensive role in stimulating immune response and playing an important role in

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activation of the adaptive immune response and tissue homeostasis [6]. THP-1 is a monocyte leukemia cell line from humans which differentiate into active macrophages after treatment with phorbol 12-myristate 13-acetate (PMA). This cell line has been used extensively to study monocyte/macrophage mechanisms, their regulatory functions, nutrient transport and signaling pathways [7].

Therefore, research comparing infection of *M.tb* to both cells (hMDMs and THP-1) are scarce. Also, whether they can be used as each other's alternative is unclear. Hence, in the present study, we are comparing hMDM and THP-1 response at various levels to mycobacterial infection. Here, for the first time we conduct a head to head comparison of both cell types using a drug-susceptible (H37Rv) and a drug-resistant strain (R179) of *M.tb* through bacterial uptake, host cell viability, mRNA expression level as well as cytokines secretion upon infection.

2. Methodology

2.1. Cells and culture medium

Human macrophage-like cells, THP-1 (ATCC-88081201), were cultured in RPMI-1640 supplemented with 10% heat-inactivated fetal calf serum (Biochrome, Germany). The cells were incubated at 37 ºC in a 5% CO_2 incubator. THP-1 cells were treated with a final concentration of 100 nM Phorbol 12-Myristate 13-Acetate (PMA; Sigma Aldrich, USA) for 48 h. In the present study, commercially available THP-1 like macrophages were used, experiments were carried out within 5 passages to avoid change of cellular properties [8]. Cells were in a healthy and stable morphological state. The morphology of cells was frequently examined under a microscope. Cytotoxicity analysis was also performed to study host cell viability.

For human monocyte derived macrophage cells, phlebotomy was performed on three healthy individuals (80 ml blood) after their written consent (in accordance with declaration of Helsinki) in Na-Heparin vacutainers. Ethical permission for the same had been obtained from the Ethics Committee, Stellenbosch University, Tygerberg campus, Cape Town (HREC Reference #S17/10/211). Healthy individuals were selected based on inclusion criteria such as no symptoms of tuberculosis including clear Chest X-Ray, night sweats and fever. Individuals on strong medication or any recent past surgery, pregnancy, anemia and insomnia were among the exclusion criteria. Whole blood from the recruited participants were further diluted with PBS (Sigma Aldrich, USA) in 1:1 ratio. This diluted ratio of blood and PBS was gently poured over the Histopaque (Sigma Aldrich, USA) layer and centrifuged at 804x*g* for 20 min. The buffy coat layer that appears between the blood plasma and the RBCs, containing the peripheral blood mononuclear cells (PBMCs) was collected in different tubes and washed twice with PBS. Total cells obtained was counted and proceeded for monocyte derived macrophages (MDM) isolation and differentiation.

Cells were cultured from human blood in Teflon jars using RPMI-1640 (Sigma Aldrich, USA) medium supplemented with 20% heparinized plasma and incubated at 37^0C, 5% CO_2 for 5 days. This allows the differentiation of monocytes into macrophages. The lids of the Teflon jars were tightened and placed on ice for 30 min. Cells were collected from Teflon jars using a Pasteur pipette. Each Teflon jar was washed and pooled into the same tube with cold RPMI-HEPES (Sigma Aldrich, USA) (4 ml per wash). The tubes were spun at 130 × *g* for 10 min at 4 ºC with no brake. The pellets were re-suspended in RPMI-glut and counted. The required volume of heparinized plasma (20% final), 10% human serum and RPMI-Glut was added to the cell suspension and plated in 24 well culture plates. The cells were incubated for 2 h at 37 ºC with 5% CO_2 for adherence of MDMs.

2.2. Bacterial strains and infection conditions

Two pathogenic *M.tb* strains R179 (drug-resistant Beijing 220 clinical isolate) and H37Rv (drug-susceptible *M.tb* strain) were selected for infection. Mycobacteria were cultured in Middlebrook 7H9 (with 10% OADC and 0.5% glycerol) without Tween 80, as the detergent is known to affect macrophage uptake and the host response to *M.tb* [9]. For infection experiments, human macrophages as well as THP-1 cells were seeded in 24-well ultralow attachment surface culture plates at 0.35×10^6 cells per well. Both cell types were infected with the two pathogenic mycobacterial strains at a MOI = 2 using the "syringe settle filtrate" (SSF) method [10] and incubated four hours for bacterial uptake. A similar uptake (measured by CFUs) of the two strains was observed in both hMDMs and THP-1 cells. The cells were then washed with phosphate buffered saline (PBS) three times to remove any extracellular mycobacteria. Cells were incubated for an additional 20/92 h in complete RPMI medium (24/96 h in total depicting early and late response to infection). Uninfected hMDMs as well as THP-1 cells served as the control/uninfected samples.

2.3. RNA extraction

Total RNA from human macrophages and THP-1 cells were extracted using the RNeasy Plus Mini Kit (QIAGEN, USA) as per the manufacturer's instructions. The extraction was performed immediately following the 24- and 96 hs infection period. The 'gDNA eliminator' column included in this kit was used to remove genomic DNA in all samples. For each experiment, RNA quantity and quality were measured using Agilent 2100 Bioanalyzer. The RNA with a high RNA integrity Number (RIN) (≥9) was used for cDNA preparation prior to quantitative real time PCR experiments.

2.4. Quantitative qPCR

For cDNA preparation 0.5 µg RNA was converted using the Quantitect ^{R} Reverse Transcription Kit (QIAGEN, USA). To ensure the removal of genomic DNA, 'gDNA wipe-out buffer' was added to RNA (included in the kit) prior to the RNA conversion step. qPCR amplification was run on a LightCycler ^{R} 96 system (Roche, Germany). LightCycler^R 480 SYBR Green I Master was used for various differentially expressed genes using QuantiTect^R primer assays with 10 µl of reaction volume. The reference genes (hsUBC and hsGAPDH) were selected conferring to stable expression levels of known cytokines. The amplification process involves 45 cycles of 95 º C for 10 s followed by 60 º C for 10 s and finally 72 °C for 10 s. Gene expression fold-changes was computed for pathogenic infected and uninfected macrophages using calibrated normalized relative quantities using the equation $N = N_0 \times 2^{Cp}$. All qPCRs were done on RNA extracted from six different experiments. All biological replicates having a positive control and a non-reverse transcription control was run in triplicate (along with calibrator) as per the MIQE Guidelines [10].

2.5. Determination of bacterial uptake and viability

Infected cells were lysed using 0.1% Triton X-100. Bacterial uptake was determined by serial dilution (10^{-1}–10^{-4}) and plating out of mycobacteria onto 7H11 agar plates. The agar plates were incubated at 37 °C for 5 weeks and CFUs/ml was determined. Bacterial survival within the infected cells was monitored at 4 h and day 1 to day 16 post-infection respectively. Fresh media was replaced after every four days of the culture.

2.6. Cytotoxicity analysis

Both hMDMs and THP-1 like-macrophages were seeded in a 24 well plate with 0.35×10^6 cells/well. Cells were maintained for 16 days post-infection. Every set of cells including uninfected and infected cells were processed at 4, 8, 12, 14, and 16 days post-infection respectively. Fresh media was replaced at every four days of culture. We did not have

a concern about cellular confluence as THP-1 cells treated with PMA leads to maturation, differentiation and a very low rate of proliferation, hence we did not experience any issue with the cellular confluence during the 16 day experiment.

Cell cytotoxicity was tested with Roche WST-1 Cell Cytotoxicity Reagent (Roche, USA) in 1:10 dilution of WST-1 reagent to RPMI complete media. Cells were incubated for 1 h at 37 º C and 5% CO_2. Absorbance was measured at 450 and 630 nm. The difference between the two absorbance readings was plotted in Excel as percentage values.

2.7. Luminex assay

Sample levels were evaluated using ThermoFisher Luminex kits 12plex PPX-12 (ThermoFisher Custom Procarta-12 Plex) on Bioplex platform (Bioplex™, BioRad Laboratories). Luminex assay was performed following ThermoFisher protocol and instructions. The assay was performed by a single technician where all samples were evaluated in duplicate. All analyte levels included in the kits which are a part of quality control reagents were within the expected ranges. Co-efficient of variation of these samples for duplicate runs did not vary a lot between analytes for both inter and intra plate. The variation range for duplicate runs was below 20% (5.2%–19.6% range). The standard curve for all samples ranged from 3.6 to 10000 pg/ml. Bioplex Manager Software version 4.1.1 was used for data analysis.

2.8. Statistical analysis

Real time qPCR data was analyzed using Light Cycler 96 SW 1.1 Software and Graph-pad Prism V7. Relative Expression of the cytokines was measured through the software in response to the Calibrator and non-transcription control. The relative expression data of the cytokines was further analyzed through Graph-pad prism to generate the p-values through One-Way ANOVA. The *p*-values were finally generated through Multiple Testing using Tukey corrections. The data (in triplicate) was finally plotted in histograms with respective mean and standard deviations. Cytotoxicity graphs and CFUs were plotted with an average of the technical triplicates leading to the mean of all the Biological replicates. Statistical analysis was performed through Graph-pad Prism V7 software where the percentage of every expressing cell was generated, and p value was calculated using One-Way ANOVA. Luminex data was analyzed by One-Way ANOVA using Graph-pad Prism V7 for Windows (Graph-pad Software, San Diego California, USA).

3. Results

3.1. Determination of colony forming units

Intracellular growth rates were determined by colony forming units per ml (CFUs/ml) over a period of 16 days (Fig. 1A and B) at 4 h post-infection, THP-1 cells were infected with 122,000 CFUs/ml of R179 and 131,000 CFUs/ml of H37Rv. Also, at 4 h post-infection, hMDMs were infected with 124,000 CFUs/ml of R179 and 132,000 CFUs/ml of H37Rv. CFUs were measured at day 0, 1, 4, 8, 12, 14- and 16-days post-infection. No difference in the mycobacterial uptake (measured by CFUs) was observed between the two cell types using R179 ($p = 0.954$) and H37Rv ($p = 0.922$). Also, there was no difference observed in mycobacterial uptake between drug-resistant R179 and drug-susceptible H37Rv *M.tb* pathogenic strains for both THP-1 ($p = 0.894$) and hMDMs ($p = 0.949$).

3.2. THP-1 and hMDM viability over-time post-infection

Cell viability was tested in both cell types which included uninfected and infected cells (Fig. 2). THP-1 cells had 90 and 88% viability at day 1 and 4 post-infection with R179, whereas had 93 and 91% viability at day 1 and 4 post-infection with H37Rv. Cell viability

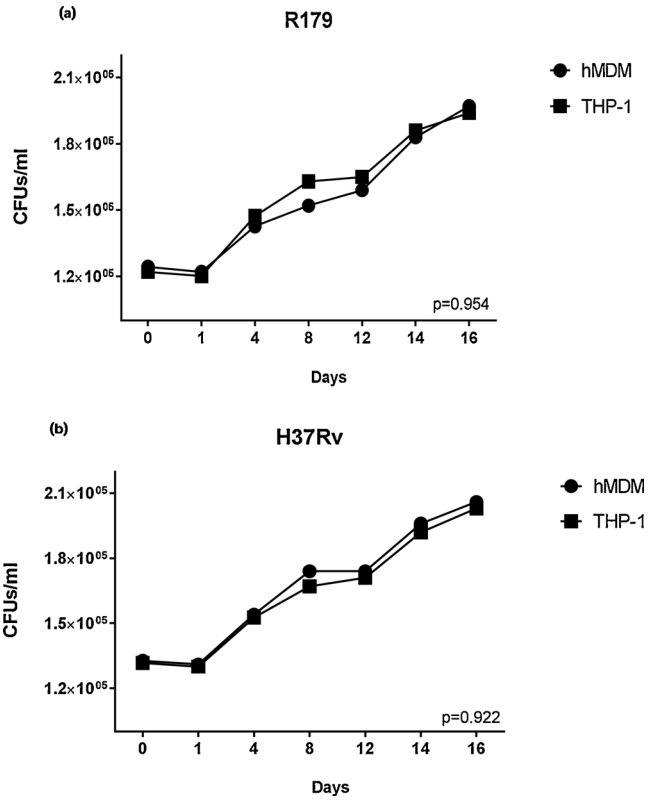

Fig. 1. Colony counts of M.tb from day 0 (at 4 h post infection) up-to day 16 post infection in hMDM and THP-1 cells (Fig. 1A and B). THP-1 cells and hMDMs are depicted with two different markers at different time points. Both culture models represent a similar trend with a gradual increase in CFUs/ml up-to day 16. The data represents the means of results from three different experiments and standard deviation of the means was calculated.

decreased slightly over time. On the other hand, hMDMs had 89 and 87% viability at day 1 and 4 post-infection with R179 and had 91 and 90% viability at day 1 and 4 post-infection with H37Rv, and like THP-1 cells, viability in hMDMs also decreased slightly over time. Thus, no significant difference in the cell viability was observed after infection with drug-susceptible and drug-resistant *M.tb* strains. It is noteworthy, that the cell viability was found similar across both cell types, indicating their life-span *in vitro* post-infection is similar, for both THP-1 and hMDMs (R179, $p = 0.271$) (H37Rv, $p = 0.168$) respectively. There was also no difference observed in viability between the susceptible H37Rv and resistant R179 *M.tb* strains for both THP-1 ($p = 0.221$) and hMDMs ($p = 0.647$).

3.3. Determination of cytokine/chemokine mRNA levels in THP-1 and hMDMs at 24 and 96 h post-infection

We assessed gene expression of the typical pro- and anti-inflammatory cytokines and chemokines induced after infection with M.tb. We did this through qPCR and assessed relative expression of the genes in uninfected and infected samples (Fig. 3a and b).

On studying relative expression of IL-6, we found that upon infection with R179, THP-1 and hMDMs had no difference at their mRNA level at both 24- and 96 hs post-infection. Upon infection with H37Rv, THP-1 cells showed higher expression at 24 h post-infection ($p = 0.036$), but no difference at 96 h post-infection.

IL-12 mRNA levels were similarly expressed at 24 h post-infection with R179 and H37Rv for both THP-1 cells and hMDMs. However, after 96 h of infection, hMDM showed relatively higher expression as compared to THP-1 cells. Upon infection with R179, hMDM had higher mRNA level expression ($p = 0.044$). hMDMs showed higher mRNA

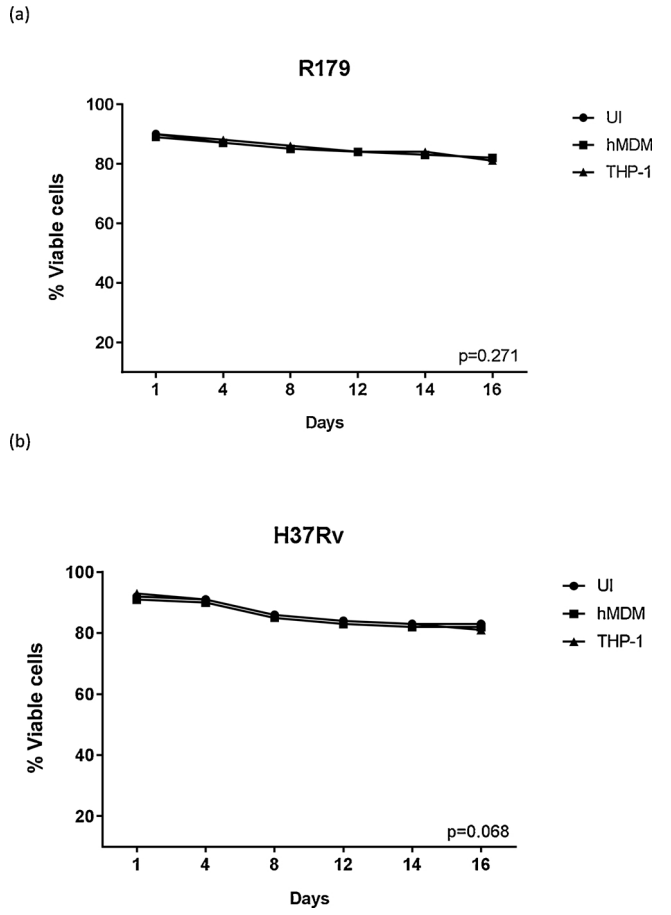

Fig. 2. Host cell viability upon infection with *M.tb* strains was measured through cytotoxicity assay from day 1 to day 16 post-infection. Histogram depicts an average of the uninfected hMDMs and THP-1 s along with infected hMDMs and THP1 cells. Fig. 2(A): Cytotoxicity analysis of cells infected with R179. Fig. 2(B): Cytotoxicity analysis of cells infected with H37Rv. The data represents the means of results from three different experiments and the bars represent standard deviation of the means.

expression than THP-1 cells upon infection with H37Rv ($p = 0.001$).

Next, we assessed the expression of the chemokines CCL2 and CCL5 upon infection. CCL2 showed no difference upon R179 infection at 24- and 96 hs post-infection. Relative expression of CCL2 in hMDMs infected with H37Rv was significantly high when compared to that of THP-1 cells at both 24- and 96 -hs post-infection (p-value < 0.001). The relative expression of CCL5 on the other hand had no difference between THP-1 cells and hMDMs after infection with both pathogenic strains at 24- and 96 -hs post-infection.

THP-1 cells and hMDMs had no difference in IL-1β expression at both time points across the strains. Interestingly, both cell types showed a strain-specific response after infection with H37Rv through significantly upregulating IL-1β expression. The trend of IL-1β mRNA expression was consistent in both cell types.

Both TNF-α and IFN-γ had increased expression after infection in both cell lines at both time points but did not show any significant differences and followed a similar level of expression. Finally, we measured the relative expression of IL-10 and observed that THP-1 cells and hMDMs had elevated levels of the mRNA throughout the 96-h infection period, whereas the THP-1 cells appeared to have an early response to infection after infecting with R179. Thereafter, expression remained similar. The relative expression of IL-10 which is a known anti-inflammatory cytokine had no major difference in the uninfected samples rather showed a significant difference in hMDM cells infected with H37Rv at 24 h when compared to THP-1 cells ($p = 0.024$). After studying relative expressions, we can conclude that there is no major difference observed in cytokines/chemokines mRNA levels between infected THP-1 and hMDMs at 24 and 96 h post infection. Although there were significant inter-strain differences in mRNA levels over-time, this did not contribute to overall significance.

3.4. Secreted cytokine levels between infected THP-1 and hMDMs at 24 and 96 h post-infection

Secreted cytokines measured using cell supernatant did not show any significant difference across the strains between the two cell lines. Luminex assay was performed and the secretory cytokines/chemokines was measured against their standards in duplicates for 24- and 96 -hs post-infection (Fig. 4). Five different cytokines including IL-6, IL-12, IL-1β, IFN-γ and TNF-α were measured through Luminex and the data was analyzed using Graph-pad Prism. The cytokines secretion had no difference when compared across the strains. It was interesting to note that all five cytokines did not show any significant difference at 24 h post-infection across the strains for THP-1 cells and hMDMs (R179, $p = 0.861$) (H37Rv, $p = 0.986$). The cytokines were also measured at 96 h post-infection, and still did not show any significant difference as late expressions across the strains for THP-1 and hMDMs (R179, $p = 0.765$) (H37Rv, $p = 0.826$). There was also no difference observed in cytokines secretion between the susceptible H37Rv and resistant R179 *M.tb* strains for both THP-1 ($p = 0.168$) and hMDM ($p = 0.272$) at 24 h post-infection and THP-1 ($p = 0.324$) and hMDM ($p = 0.296$) at 96 h post-infection.

4. Discussion

In the present study, we have used two types of macrophage cells (hMDMs and THP-1) to study host response towards *M.tb* infection, both these cell types are widely used to study associations at the host-pathogen interface [12]. We also focused on comparing a drug-susceptible and a drug-resistant *M.tb* strain (H37Rv and R179), hence to give a clear picture for type of infection and host response. We carefully selected six cytokines and two chemokines for monitoring mRNA expression level through qPCR (IL-6, IL-12, IL1β, IFN-γ, TNFα, IL-10, CCL2 and CCL5). We also measured five signature cytokines (IL-6, IL-12, IL1β, IFN-γ and TNFα) among the above eight for studying protein secretion through Luminex. The selected panel of cytokines and chemokines were reported to play specific roles in context of *M.tb* infection [13]. Importantly, in the present study, we have avoided any external stimulation, including cell isolation through bead separation, LPS or any cytokine stimulation such as IFN-γ, hence minimizing any alteration in surface receptors or cytokines expression, which was lacking in previous studies. There are studies which include activation factors for cellular differentiation and isolation. Bead separation or magnetic activated cell sorting methods for macrophage isolation and other chemicals-supplementation for macrophage differentiation and intracellular growth are used while reviewing variants of *M.tb* [14,15].

The main findings of the present study is as follow: 1) we found similar uptake of bacteria in both cell types (hMDMs and THP-1) at various time points (up to 16 days) post-infection, both cell types had similar response with drug-susceptible (H37Rv) and drug-resistant (R179) pathogenic *M.tb* strains, 2) the viability of both cell types were found to be similar at various time points (up to day 16) post-infection, 3) comparison of the mRNA expression level between the two cell types before and after infection (24 and 96 h), revealed no difference in signature cytokines (IFN-γ, IL-1β and TNF-α) and chemokine (CCL5), though some of the cytokines/chemokines differed across both cell types and 4) comparison of secreted cytokines levels before and after infection of both cell types, were found to be similar at both time points (24 and 96 h) across the strains.

Previous studies investigated the comparison of primary and secondary cell lines including blood macrophages and THP-1 like

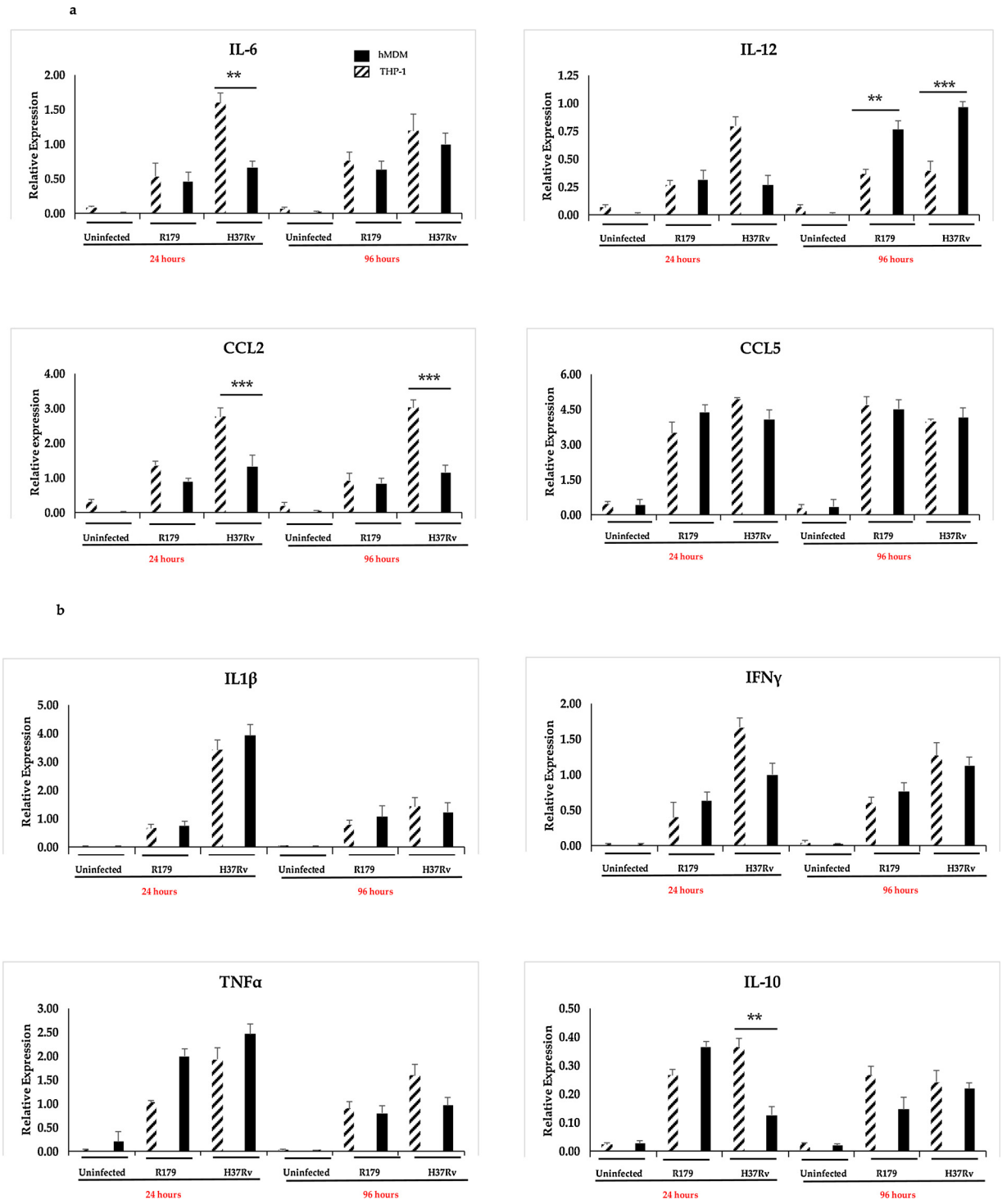

Fig. 3. (a&b): qPCR-based analysis of pro- and anti-inflammatory cytokines and chemokines in hMDM and THP-I cells after 24 and 96 h of infection with *M.tb*. Relative mRNA expression (fold change) of various cytokines and chemokines induced by human cells following infection with two *M.tb* strains (R179 and H37Rv) as analyzed through qPCR (n = 3). GAPDH and UBC used as reference genes. Standard deviation is shown by error bars. One-way-ANOVA with Tukey correction for multiple comparisons was used to determine p values depicted as (*) where ** = $p < 0.05$, *** = $p < 0.001$.

macrophages for bacterial uptake measured by colony forming units. They showed both primary and secondary macrophages CFUs were similar at resting phase *i.e.* without any activation or stimulation [1]. Another study showed no difference in bacterial uptake in THP-1 and hMDMs in a control state. But, upon stimulation with p19 (19-kDa *M. tuberculosis* glycoprotein), a significant reduction in CFU recovery was noted. This reduction was still similar in both the cell types indicating that there is minimal or no significant difference in *M.tb* infected hMDMs and THP-1 cells [16]. In the present study, we avoided any kind of stimulations that could alter surface receptors. Moreover, we performed head-to-head comparison of CFUs for hMDMs and THP-1 which showed similar CFUs at various time points (up to day 16 post-infection) for both susceptible and resistant mycobacterial strains (R179, p = 0.954 and H37Rv, p = 0.922). Results indicated, both cell types had similar bacterial uptake (similar CFUs), this has useful implications, as THP-1 cells can be used as an alternate to human blood macrophages

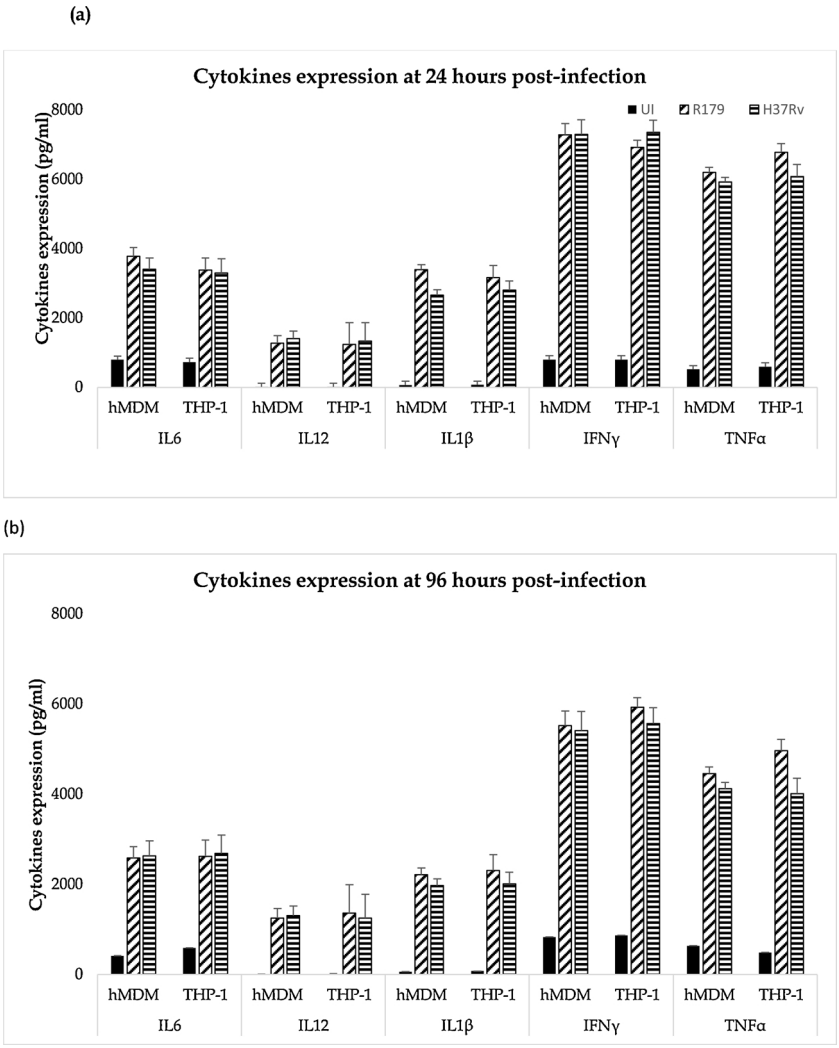

Fig. 4. (a&b): Cytokines secretion through Luminex. Luminex was performed in both cell lines hMDMs and THP-1 s upon 24 and 96 h post-infection with H37Rv and R179 *M.tb* strains. The data represents the means of results from three different experiments and the bars represent standard deviation of the means.

in *in-vitro* experiments encompassing *M.tb* infection (either susceptible or resistant).

As tissue macrophages originating from circulating mononuclear monocytes do not need to self-renew, hence the hMDMs do not proliferate *in-vitro* [16]. Similarly, treating THP-1 cells with PMA leads to maturation, differentiation and a very low proliferation rate [17–19]. In the present study, we cultured hMDMs and THP-1 cells up-to day 16 post-infection to study the intra-cellular bacterial growth and the host cell viability. During the 16 days of the experiments no extracellular bacterial growth was detected by regular visual inspections using the microscope. Fresh media was replaced after every 4 days of culture. Luminex assay was performed at 24- and 96 -hs post-infection (without any media replacement), hence not effecting the cytokine secretion into the cell supernatant during this period.

In the present study, host cell viability of both cell types analyzed by a cell cytotoxicity assay proved to be informative for *in-vitro M.tb* infection experiments. It was essential to study the uninfected and infected cell viability over time post-infection. Both cell types at MOI of 2 had $\leq$85% viability up to day 8 post-infection and gradually decreased by day 16 following the same trend. Previous study showed difference in cell viability in the two cell lines but at higher MOI (10 and 100). They reported that upon *M.tb* infection, hMDMs had higher cell death compared to THP-1 at MOI of 10 and 100. But, they also showed that at MOI of 1, there was no significant change in the cytotoxicity between the two cell lines [21]. Present study agrees with the results of the previous study, and the viability of both cell types are similar over 16 days post-infection, indicating both cell types have similar survivability under infection.

We determined the host response of both cell types by quantifying mRNA expression by qPCR and determination of secreted levels of cytokines and chemokines using Luminex. qPCR is considered to have high sensitivity and accuracy for gene expression quantification [22]. We carefully selected a panel of six cytokines and two chemokines which are known to play significant role in *M.tb* infection.

We found three cytokines (IFN-γ, IL1β, and TNFα) and one chemokine (CCL5) to be non-significant between the two cell types at two time points (24 and 96 h post-infection) across both strains. IFN-γ, is a known signature cytokine in *M.tb* infection and regarded as crucial cytokine to fight against *M.tb* [23,24]. IFN-γ showed a similar gene expression by qPCR in both cell types after infection with both susceptible and resistant strains. Also, we found similar level of secreted IFN-γ (detected by Luminex assay) from both cell types across susceptible and resistant strains, hence, confirming the qPCR findings. It is important to note that IFN-γ consistently remained similar in both cell types, indicating a similar host response from both cell types.

IL-1β did show an early response at 24 h in both the cell types upon H37Rv infection, though similar in both cell types. The expression reduced at later time point (96 h post-infection) and consistently remained similar in both cell types. The secreted levels of IL-1β, determined by luminex assay was found to be similar in both cell types at

24- and 96 hs post-infection. IL-1β is regarded as crucial cytokine for survival against *M.tb* infection, previous studies have shown that IL-1β knock-out mice were more susceptible to *M.tb* infection [25,26]. Other important cytokine was TNF-α, which is believed to be responsible in apoptosis of *M.tb* infected cells *in-vitro* [27,28]. Further, secreted levels and mRNA expression of TNF-α was also found to be similar in both cell types at 24 and 96 h, these results are in agreement with a the previously published study [1].

We also measured IL-6, which was shown to be regulated by *M.tb* to inhibit type I interferon signaling and, consequently, disease progression in TB [29]. It was also reported that IL-6 secreted after infection of macrophages with *M.tb* inhibits the responses of uninfected macrophages to IFN-γ [30]. In the present study, the mRNA levels of IL-6 was found to be significantly higher in THP-1 cells as compared to hMDMs at 24 h post-infection with H37Rv, though the expression level were found to be similar after 96 h. This indicated an early response in THP-1 cells after infection with susceptible *M.tb* (H37Rv). The expression level of IL-6 was found to be similar in both cell types at 24 and 96 h after infection with R179. Since, the secreted levels of IL-6 measured through luminex was found to be similar at both time points across both *M.tb* strains, this indicated that even though the mRNA levels were higher in THP-1 cells at 24 h, the secreted levels of IL-6 were still found to be similar.

We measured IL-12 which is known to have agonist and protective role in mycobacteria infection [31,32]. We found significantly higher IL-12 in hMDMs when compared with THP-1 cells across the two pathogenic strains at 96 h. On the other hand, mRNA levels of IL-12 was found to be similar at 24 h. IL-12 is known as an essential marker for survival after *M.tb* infection [13]. But, alike IL-6, we found similar secreted levels of IL-12 from both cell types at 24 and 96 h post infection with susceptible and resistant *M.tb* strains, indicating similar host response of both cell types.

Previous study shows the relation of IL-10 with *M.tb* infection. *M.tb* infected THP-1 cells is known to induce IL-10 gene expression [33]. We, therefore measured IL-10, a known anti-inflammatory cytokine where THP-1 had higher expression upon H37Rv infection at 24 h when compared with hMDMs, however, there was no difference at later time point (at 96 h). The cytokine secretion of IL-10 was found to be similar for both cell types at 24 and 96 h after infection with R179.

A known chemokine CCL2 showed higher expressions in THP-1 cells at both early (24 h) and late (96 h) post H37Rv infection. However, the expression level of CCL2 after infection with R179 was found to be similar for both cell types at 24 and 96 h. CCL2 is known to maximize and organize early macrophages in the lungs, which was strongly depicted by THP-1 cells as compared to hMDMs. Chemokine CCL5 showed similar mRNA expression throughout (24 and 96 h post-infection) in both the cell types across the two strains. CCL5 is speculated to enhance macrophage *M.tb* killing and facilitate early dendritic cell accumulation in the lymph node [13].

An overall similar host response (measured by cytokines/chemokines) at mRNA levels across the two pathogenic strains in both cell types was observed. There are some minor variations in mRNA levels, particularly after infection with susceptible strain (H37Rv), but this did not result in any variation observed in mycobacterial uptake, cellular viability as well as host response towards cytokines secretion. Host response towards mRNA expression was found to be consistently similar after infection with resistance strain R179 as compared to the susceptible H37Rv.

Overall, we observed a similar trend in both the cell types without any notable significant differences between the two. The results therefore validate the utility of THP-1 cells to study *M.tb* infection and are comparable to the hMDMs response to infection, *i.e.* THP-1 cells behave like native human monocyte derived macrophages with regards to the parameters measured in this study. As an in-depth study to relate the physiological functions exhibited by these cells, THP-1 cells prove to be a valuable model exploring macrophage specific genes. Due to their similarity and relatively similar behavior with native cells, they have proven to be a valuable model for macrophage differentiation mechanisms.

Our study has some limitations, we only used two pathogenic *M.tb* strains. Though our motive was to compare both susceptible and resistant strains, we will compare more strains in future studies. Also, we have measured the CFUs and viability of cells up to day 16, thus, we cannot conclude what happens to bacterial uptake, viability and host response at later time points. Moreover, we are fully aware that we should investigate other host response signatures (with broad panel of cytokines and chemokines).

We therefore conclude that both cell types, *i.e.* hMDMs and THP-1 have shown similar bacterial uptake (measured by CFUs), cellular viability and similar host response signature biomarkers to both drug-susceptible (H37Rv) and drug-resistant (R179) mycobacterial infection.

Authors contributions

AM, HM and BB designed the experiments. AM, GL and ZM performed the experiments. AM and HM analyzed the data. AM, HM, BB and GL wrote and edited the manuscript.

Declaration of Competing Interest

The authors declare that no conflict of interest exists.

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