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Original Article

Gene Silencing of Toll-like Receptor 2 Gene Expression as a Tactic to Control Mycobacterium Tuberculosis and Granuloma Formation

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Abstract

Mycobacterium tuberculosis (MT) is the causative agent of tuberculosis (TB) in humans. Tuberculosis is one of the top 10 causes of mortality worldwide, resulting in 1.8 million deaths and 10.4 million new cases in 2016. Understanding the fundamental features of MT biology is critical to the eradication of MT in the future. Due to the increasing frequency of antimicrobial treatment resistance and problems in vaccine development, the pathogenesis of TB for its survival and growth is highly dependent on host lipids and stimulated-lipid droplets formation. Toll-like receptor 2 (TLR2) forms heterophilic dimers with TLR1 and TLR6, therefore, recognizing many MT components. Both of these receptors identify the invading antigen and activate downstream protein kinases. Some studies demonstrated that the cyclooxygenase-2 (COX-2) promoter-driven gene expression includes connecting sites for transcription factors, such as nuclear factor-kappa B, CREB, NFAT, and c/EBPβ. The current study aimed to investigate the role of the TLR2 receptor in positively regulating prostaglandin E2 production in *M. bovis* (BCG) infected macrophages *in vivo* using a human monocytic cell line THP-1. Our results revealed that MT infection triggers a time-dependent increase in COX-2 expression via pathways involving TLR2 receptor activation and enhances COX-2 expression, leading to an increase in lipid droplet formation and suppression of macrophage activation.

Keywords: BCG, THP-1 cells model, PPARγ, TLR2 siRNA knockdown

1. Introduction

Mycobacterium tuberculosis (MT) is an agent, a highly successful pathogen of tuberculosis (TB), that infects the human population, and it has adjusted to growth within the macrophage environment. *Mycobacterium tuberculosis* has developed various ways to damage the metabolic and immune responses of the host cells. To evade inflammatory cells, these pathogenic mycobacteria species activate and regulate many genes within the host cells, including host lipid metabolism, controlled by intra-cellularly induced genes via MT (1).

The pathogenesis of TB is entirely dependent on host lipids for survival and growth, and it has stimulated the formation of lipid droplets, which are significant in TB and leprosy, such as foam-like macrophages (2). The toll-like receptors (TLRs) are proteins that play an essential role in fighting the invading microorganisms and triggering the innate immune responses. The TLRs can recognize the pathogen-associated microbial patterns of microbes or damage-associated molecular pattern intermediates of the cell debris (1). The recognition of MT is mediated by different groups of pattern recognition receptors, including TLRs, Nod-like

receptors (NLRs), C-type lectin receptors (CLRs), and TLRs family members, such as TLR2, -4, and -9 with the adaptor molecule MyD88, which have molecules of importance in the immediate immune response to MT infection (2). The re-sensitization of macrophages to assaulting pathogens is based on the upraised expression of TLR2 and mCD14 genes (3). Because the TLR2 receptor forms heterophilic dimers with other receptors like TLR1 and TLR6, together are functionally attached to TLR2 on macrophage surfaces and recognize MT triacyl and diacyl lipopeptides, respectively (4). Moreover, polymorphisms in the TLR2 gene may increase the risk of MT vulnerability. When challenged with gram-positive bacteria, impairing TLR2 functions in murine phagocytes via gene knockout or blocking antibodies eliminates TNF- and IL-6 (1).

Tuberculosis interferes with the maturation of phagocytosis and prevents subsequent antigen emergence upon escaping from lysis by lysosomal hydrolysis. To prevent the mycobacteria from surviving, the host macrophages enhance the autophagy process through the maturation of the mycobacterial phagosome. In addition, the rising cyclooxygenase-2 (COX-2) expression reinforces the autophagy machinery in macrophages as a crucial bactericidal mechanism (5). Prostaglandin E2 (PGE2) exerts an immunosuppressive effect in the situation of mycobacterial invasion. Due to COX-2 being a rate-limiting enzyme in the biosynthesis of PGE2, it is necessary to define the mechanisms by which MT regulates COX-2 expression in macrophages (5). Previous research has shown that the nuclear factor-kappa B (NF-κB) and cAMP response element (CRE) of the COX-2 promoter are significant to *M. avium*-induced COX-2 gene expression. Signals triggered by *M. avium* are originated in the TLR2 receptor. AGE-albumin, a prototypic ligand, activated p21(ras) binds to TLR2 and activates extracellular signal-regulated kinase (ERK). In contrast, tumor necrosis factor receptor-associated factor 6, in turn, stimulates transforming activated growth factor kinase 1 stimulates p38 mitogen-activated protein kinase (MAPK), both ERK and p38 MAPK activation converge to regulate the activation of mitogen- and stress-activated kinase 1 (MSK1) (5). The MSK1 mediates the stimulatory effect on CRE-dependent gene expression and is also necessary for *M. avium*-induced NF-κB-dependent gene expression, whereas it is insufficient for COX-2 promoter-driven gene expression.

Tuberculosis and its cell walls ingredients such as lipomannan, lipoarabinomannan (LAM), and proviral integrations of moloney virus (PIM) induced IL-8 mRNA expression and protein secretion *in vitro* from the alveolar macrophages. Some cases mentioned that the lipopolysaccharide, phorbol myristate acetate, reactive oxygen species, IFN-γ, IL-1, IL-6, and TNF-α induced IL-8 expression up to 100-fold (6). Furthermore, it is shown that *M. bovis* (BCG) infection promotes the expression of peroxisome proliferator-activated receptor gamma (PPARγ) via TLR2 in rat macrophages and controls the lipid-body formation and PGE2 biosynthesis (7).

2. Materials and Methods

2.1. Preparation of Cell Culture Media

A complete cell culture medium was prepared by mixing the following ingredients: 8.2 of RPMI 1640, 10 g ($NaCO_3$), 500 ml (T.D.W), 50 ml (FBS, 10 %), and 0.2 mM L-glutamine. The components were mixed, stirred, and then sterilized by filtration with a syringe filter of 0.22 μm. After filtration, the prepared antibiotics were added, including 100 U/ml penicillin and 100 g/ml streptomycin at 37◦C in a humidified atmosphere containing 5% CO_2. The THP-1 cells were maintained in a culture medium and seeded in 12-well plates, grown to 80% confluence, and transfected with the constructs using (lipofectamine) DNA-fectin TM Plus based on the manufacturer's instructions.

2.2. Short Interfering RNA (siRNA) and Transfection

The TLR2 siRNAs targeting three were designed by (abm. Cat. No. G487, CANADA). According to the

manufacturer's instructions, the siRNA was transfected into cells using (lipofectamine) DNA-fectin TM Plus transfection reagent. Each siRNA oligo was dissolved with 62.5 µl of DEPC water to prepare 20 µM. Each siRNA was mixed with1.2 µl of lipofectamine and left for 20 min before adding the mixture to the cells. In parallel, 62.5 nmole of the negative control was combined with 1.2 µl of lipofectamine.

To silence the expression of TLR2, macrophages were transfected with the TLR2 siRNA. The siRNA transfection was performed according to the manufacturer's instructions. In summary, THP-1cells were incubated in the siRNA transfection medium (abm .Cat. No. G487, CANADA) at a density of 2×10^6 cells/well in 12-well cell culture plates, followed by the addition of the TLR2 siRNA or negative control siRNA, and incubated at 37◦C for 4 days, the transfection efficiency of >95% was demonstrated by hemocytometer (Superior. Germany). Each well contained 1ml medium, 0.8 µg DNA (µg), 2µl DNA-fectin TM Plus (µl), and 100µl transfection medium. Then, the BCG vaccine at a concentration of 8×10^6 cells/ml was added to six wells, as opposed to six wells without BCG vaccine. The 12-well plates were incubated at 37◦C for 4 h and were harvested later.

2.3. RT-qPCR

Total RNA from the macrophages was extracted using an RNA extraction kit (Promega, USA) based on the manufacturer's instructions. The extracted total RNA was quantified by absorbance at 280 nm using a NanoDrop 2000c Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The mRNA in the total RNA was reverse-transcribed to complementary DNA using a cDNA synthesis kit (Promega, USA). In addition, the qPCR was performed in the I Cycler iQ5 (Bio-Rad) using SYBR RTpermix (England) under the following conditions: 10 sec at 95°C, 45 cycles of 15 sec at 95°C, and 30 sec at 59°C. The mRNA expression levels, which were normalized against β-actin, were calculated and expressed as $2^{-\Delta\Delta CT}$. The primers used for qPCR were as follows: β-actin; F- 5′-GATTACTGCTCTG GCTCCTAGC-3′ and R- 5′-GACTCATCGTACTCCTGCTTGC-3′ and for TRL2: F-5′-AAG AGGAAGCCCAAGAAAGC-3′and R- 5′-CAATGG GAATCCTGCT CACT-3′and for COX-2; F 5′-GAAGCCTTCTCCAACCTCTCCTA-3′ and R- 5′-CCCAGGTCCTCGCTTATGATCT-3′.

3. Results

3.1. Relative Gene Expression of TLR2 in TLR2-siRNA Knockdown without BCG Vaccine

To gauge the TLR2 gene expression that plays a vital role in the recognition of endogenous and exogenous ligands, the templates of cDNA were used in qPCR relative expression assay with a master mix of SYBR green and TLR2-specific primers, the averages and standard deviation were considered for three replicates. The findings showed that TLR2 gene expression was high in non-TLR2 knockdown control as compared with the TLR2 knockdown samples (Figure 1A). The results were depended on ΔΔ CTs analysis after discounting the housekeeping gene (β-actin). Furthermore, to precisely check the fold change, the values of all samples were normalized to 1 by fold change analysis, and their values in TLR2 knockdown samples were compared to that 1. Our results demonstrated that the TLR2 relative expression was reduced to 99% due to the TLR2 siRNA knockdown (Figure 2A).

3.2. Relative Gene Expression of TLR2 in TLR2-siRNA Knockdown plus BCG Vaccine

The averages and standard deviation were considered for three replicates. The results showed that TLR2 gene expression was high in non-TLR2 knockdown control plus BCG vaccine as compared to the TLR2 knockdown plus BCG vaccine samples (Figure 1B). The findings were dependent on ΔΔ CTs analysis after subtracting the β-actin, the values of all samples were normalized to 1 by fold change analysis, and their values in the TLR2 knockdown plus BCG were compared to normalized 1. Our results demonstrated that the TLR2 relative expression was reduced to 56%

one-fold due to the siRNA knockdown plus BCG vaccine (Figure 2B) after only 4 h of BCG challenge.

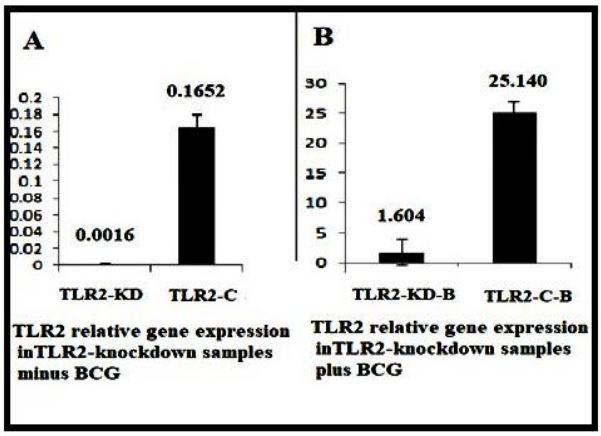

Figure 1. A: Toll-like receptor 2 (TLR2) relative gene expression in TLR2 knockdown samples (TLR2-KD) and non-TLR2 knockdown control (TLR2-C) samples. All RNA was extracted and reverse-transcribed and the synthesized cDNA was used as a template for qPCR relative expression assay using SYBR green master mix. The averages and standard deviation were considered for three replicates. The TLR2 relative expression was reduced to 99% due to the siRNA knockdown minus BCG challenged. **B:** The TLR2 relative gene expression in TLR2 knockdown plus BCG (TLR2-KD-B) and non-TLR2 knockdown plus BCG (TLR2-C-B)) samples. The TLR2 relative expression was increased to 1.6% due to the siRNA knockdown plus BCG challenged, after only 4 h of BCG addition

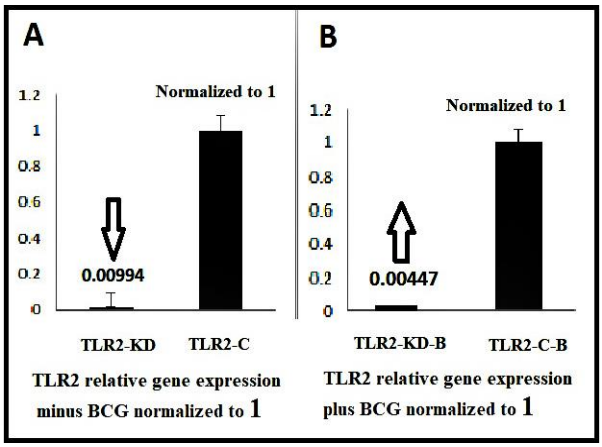

Figure 2. A: Data were analyzed by ΔΔ CTs and normalized to the housekeeping gene (β actin), which was equalized to 1. The Toll-like receptor 2 (TLR2) relative expression was reduced to 99% in TLR2-knockdown samples minus BCG (TLR2-KD) compared with control**, B:** Data were analyzed by ΔΔ CTs and normalized to the housekeeping gene (β actin), which was equalized to 1. The TLR2 relative gene expression was reduced to 56%-one-fold in TLR2-knockdown samples plus BCG vaccine (TLR2-KD-B)

3.3. Relative Gene Expression of COX-2 in TLR2-siRNA Knockdown without BCG Vaccine

Due to the role of COX-2 as an immune-modulator in MT infection, the present study aimed to evaluate its expression with the TLR2 knockdown. Consequently, total RNA was extracted and reverse-transcribed, and the synthesized DNA was used as a template for qPCR relative expression assay using SYBR green master mix. The averages and standard deviation were considered for three replicates. The data showed that the gene expression of the COX-2 gene in samples was reduced to 93%-fold, after only 4 h of BCG addition, due to TLR2 siRNA knockdown as compared to the non-TLR2 knockdown control samples (Figure 3A). The results were analyzed by 2-ΔΔ CT analysis and normalized to β-actin. The values of all samples were normalized to 1 by fold change analysis, and their values in the TLR2 knockdown with its control were compared to normalized 1. Our results revealed that the COX-2 relative gene expression was reduced to 93%-fold due to the TLR2 siRNA knockdown, as shown in (Figure 4A).

3.4. Relative Gene Expression of TLR2 in TLR2-siRNA Knockdown Plus BCG Vaccine

In the context of BCG vaccination (TLR2-B-KD) sample, total RNA was extracted, reverse-transcribed, and the synthesized DNA was used as a template for qPCR relative expression assay using SYBR green master mix. The cDNA templates were used in qPCR SYBR green master mix using COX-2 -specific primers. The expression level of the COX-2 gene was found to be remarkably higher in TLR2 knockdown plus BCG vaccine samples (Figure 3B), during only 4 h of BCG challenged as compared to the non-TLR2 knockdown control plus BCG vaccine samples. The results were analyzed by ΔΔ CT analysis and normalized to β-actin. The values of all samples were normalized to 1 by fold change analysis and their values in the TLR2 knockdown with its control were compared to normalized 1. Our results showed that the COX-2 relative expression was increased to 3 -fold in

the TLR2- siRNA knockdown plus BCG after 4 h of BCG challenged (Figure 4B).

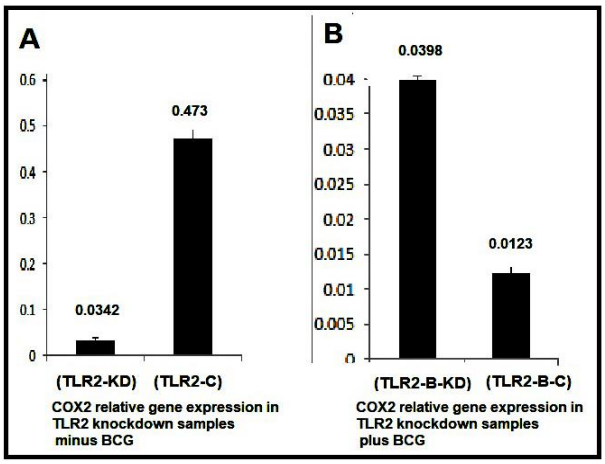

Figure 3. A: cyclooxygenase-2 (COX-2) relative gene expression in TLR2 knockdown (TLR2-KD) samples and non-TLR2 knockdown control (TLR2-C) samples minus BCG. All RNA was extracted and reverse-transcribed and the synthesized cDNA was used as a template for qPCR relative expression assay using SYBR green master mix. The averages and standard deviation were considered for three replicates. The COX-2 relative gene expression was reduced to 96% in TLR2 knockdown samples (TLR2-KD) due to the siRNA knockdown minus BCG challenged compared with its control, **B:** the COX-2 relative gene expression in (TLR2-KD-B) and (TLR2-C-B)) samples plus BCG challenged, it was increased to 1.6% due to the siRNA knockdown plus BCG challenged, after only 4 h of BCG addition

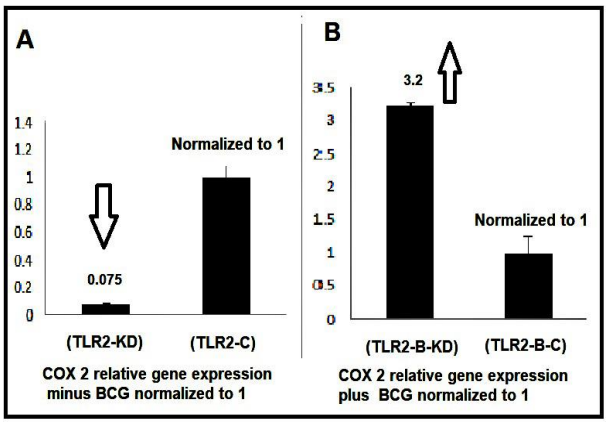

Figure 4. A: Data were analyzed by ΔΔ CTs and normalized to the housekeeping gene (β actin) which was equalized to 1. The COX-2 relative gene expression was reduced to 93% one-fold in TLR2 siRNA knockdown samples minus BCG (TLR2-KD) compared with control**, B:** Data were analyzed by ΔΔ CTs and normalized to the housekeeping gene (β actin), which was equalized to 1. The COX-2 expression was increased to 3.2-fold in the TLR2-siRNA knockdown plus BCG vaccine samples

4. Discussion

The TLR2, -4, and -9, with their adaptor molecule MyD88, are the best members of the TLR family. These receptors are an essential part in providing immunity against MT. Moreover, the TLR2 receptor can recognize MT components (2).

Based on the present study's findings, the TLR2 gene expression was reduced to 99% in TLR2 knockdown samples, and no BCG vaccine was challenged due to the siRNA knockdown (Figure 1A). However, the gene expression of TLR2 was reduced to 56% in siRNA knockdown samples challenged with the BCG vaccine (Figure 2B). This means that the macrophages (THP-1) cells in our study have knockdown in their TLR2 receptors, while TLR2 expression still exists at about 44% of total expression, indicating the up-regulation level of TLR2 gene expression in siRNA knockdown samples with BCG challenged after 4 h of BCG addition.

The high concentrations of LPS and synthetic lipid A, as well as mycobacterial cell wall fractions - lipoarabinomannan–peptidoglycan complex, all these components cause the up-regulation of TLR2 gene expression and operate with NF-κB activation into macrophages response *in vitro* and *in vivo* through a dose- and time-dependent manner (1, 8). The activity of a Drosophila Toll protein whose expression is controlled via an immunological challenge through a similar mechanism functioning in NF-kB stimulation appears to be comparable with the up-regulation of TLR expression in human and animal organisms after pathogen exposure (9). It has been demonstrated that TNF-α is sufficient to induce TLR2 expression in primary microglia. Although recent studies considered a role for MAPK, SP-1, and NF-κB signaling pathways in regulating TLR2 expression in monocytes/macrophages (10), prior reports mentioned that the TLR2 regulates at the transcriptional level in monocytes after LPS-stimulated. In addition, they found the TLR2 levels on monocytes surfaces were not seriously influenced by TLR2 knockdown with BCG

after 4 h (2). This result was consistent with Xiong, Wen (5) reports. After longer exposure to LPS-stimulated, the TLR2 levels increased (11). A new study found that in adipocytes, after a half-time (3–3.5 h), a new expression of TLR2 is transmitted inside the cell and then rises on the surface (5). Therefore, our results were consistent with these findings, owing to the TLR2 knockdown samples, which spent only 4 h with BCG challenged (Figure 4B).

The current study revealed that the gene expression of COX-2 was too low in the TLR2 siRNA knockdown and was not infected with BCG after 4 h (Figure 3A). It was reduced to 93% one-fold in samples. This decrease in gene expression of COX-2 in the TLR-2 siRNA knockdown samples, which were not BCG challenged, is the typical result since COX-2 is an inducible enzyme that is usually absent in a broad extent of normal cells and uninfected macrophages until macrophages are challenged with MT (12). In addition, our results showed an increase in gene expression of COX-2 about 3-fold in the TLR2 siRNA knockdown samples with BCG challenged after only 4 h (Figure 3B). Tuberculosis in humans and mice shows that the expression of tumor necrosis factor and interferon during inflammation is based on stopping COX-2 induced (5). It has been suggested that *M. avium* stimulates TLR4-mediated NF-κB activation, leading to COX-2 gene expression. Moreover, they proved that TLR2 participates in the induction of the COX-2 gene by the *M. avium* (5).

The latest evidence indicates the infection of macrophages with either *M. bovis* or MT; consequently causing a time-dependent increase in the expression of PPARγ in macrophages *in vitro* and *in vivo* through mechanisms that include pattern recognition receptor activation, BCG, and Man LAM. These mechanisms are essentially based on TLR2 signaling to a raised expression of PPARγ, which was evident as early at 2 h and then high levels within 24 h following BCG infection (7).

The PPARγ expression may be related to tuberculosis virulent regarding the nonpathological *M. smegmatis* failure in up-regulation of PPARγ expression. Therefore, the *M. bovis*-killing macrophage was enhanced via PPARγ inhibition, and BCG perhaps induced PPARγ activity as a run-away method (7). Furthermore, it has been demonstrated that anti-inflammatory cytokines, such as IL-4, -13, and -10, can block COX-2 expression. Additionally, the pleiotropic cytokine TGF-β can either increase or block COX-2 expression depending on the cell type (13).

Some studies demonstrated that the COX-2 promoter-driven gene expression includes connecting sites for transcription factors (e.g., NF-κB, CREB, NFAT, and c/EBPβ), and the PPARγ expression was found in mice with TLR2-deficiency. However, these mice had repressed PGE2 synthesis (14). The transcriptional regulation of the COX-2 gene is under the control of NF-κB transcription factors which prevent any over expression of COX-2, which is associated with many tumor cell lines and cancers (5). According to the aforementioned studies, which were in line with our results, we can prove the vital role of the TLR2 receptor in the activation of COX-2 promoter-driven gene expression. Since the COX-2 expression needs two transcription factors, one of them relay on TLR2 receptor signaling, either a homodimer or heterodimer 1 and TLR6 receptors and owing to have a vital role in recognizing MT and its cell wall components and second factor in pro-inflammatory cytokines (e.g., TNF-α and IFN-γ). Conversely, a decrease was observed in pro-inflammatory cytokines, such as TNF-α and IFN-γ, which is a better evidence of our results' promotion (5).

We can suggest that TLR2 siRNA knockdown may affect COX-2 gene expression either directly by preventing over expression or indirectly by reducing pro-inflammatory cytokines that are harmful to the cells. The COX-2 expression is usually transient, depending on cell type and stimuli, and its expression can be rapidly stimulated by bacterial endotoxin (such as LPS), cytokines (e.g., IL-1, -2, TNF-α), growth factors, and the tumor promoter phorbol myristate acetate (15). Increased

concentration of PGE2 during TB infection results in disease progression due to down-regulation of cell immunity. Moreover, PGE2 is thought to have an immunosuppressive function in case of TB, and it also inhibits the secretion of interferon and interleukin-2, which are important in the activation of T cells and macrophages (5).

5. Conclusion

The TLR2 signaling pathway is beneficial to MT survival. Since, by releasing inflammatory cytokines, this extended immune response generates favorable conditions for MT survival in macrophages. This condition attracts immune cells, resulting in the creation of granulomas. Therefore, TLR2 promotes the survival of MT in macrophages and its long-term endurance. As a result, TLR2 expression in THP-1 cells was consistent with our goal of reducing TLR2 expression. Our results showed a decrease in gene expression of COX-2 in the TLR2 siRNA knockdown samples minus BCG challenged after only 4 h, which in turn, reduced a synergistic effect in necrosis macrophages.

Recommendations

Further laboratory experiments regarding the role of various receptors on macrophages cells (e.g., CLRs and NLRs, and mannose receptors) in recognizing MT and studying their role in granuloma formation are required.

Authors' Contribution

Study concept and design: M. A. N.
Acquisition of data: M. A. N.
Analysis and interpretation of data: M. A. N.
Drafting of the manuscript: M. A. N.
Critical revision of the manuscript for important intellectual content: M. A. N.
Statistical analysis: M. A. N.
Administrative, technical, and material support: M. A. N.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Zhang Z, Sheng H, Shao J, Beauchamp RD, DuBois RN. Posttranscriptional regulation of cyclooxygenase-2 in rat intestinal epithelial cells. Neoplasia. 2000;2(6):523-30.
2. Ozato K, Tsujimura H, Tamura T. Toll-Like Receptor Signaling and Regulation of Cytokine Gene Expression in the Immune System. Biotechniques. 2002;33(4):66-75.
3. Wang L, Zuo M, Chen H, Liu S, Wu X, Cui Z, et al. Mycobacterium tuberculosis Lipoprotein MPT83 Induces Apoptosis of Infected Macrophages by Activating the TLR2/p38/COX-2 Signaling Pathway. J Immunol. 2017;198(12):4772-80.
4. LaPointe MC, Isenovic E. Interleukin-1beta regulation of inducible nitric oxide synthase and cyclooxygenase-2 involves the p42/44 and p38 MAPK signaling pathways in cardiac myocytes. Hypertension. 1999;33(1 Pt 2):276-82.
5. Xiong W, Wen Q, Du X, Wang J, He W, Wang R, et al. Novel Function of Cyclooxygenase-2: Suppressing Mycobacteria by Promoting Autophagy via the Protein Kinase B/Mammalian Target of Rapamycin Pathway. J Infect Dis. 2018;217(8):1267-79.
6. Shinohara T, Pantuso T, Shinohara S, Kogiso M, Myrvik QN, Henriksen RA, et al. Persistent inactivation of macrophage cyclooxygenase-2 in mycobacterial pulmonary inflammation. Am J Respir Cell Mol Biol. 2009;41(2):146-54.
7. Almeida PE, Carneiro AB, Silva AR, Bozza PT. PPARgamma Expression and Function in Mycobacterial Infection: Roles in Lipid Metabolism, Immunity, and Bacterial Killing. PPAR Res. 2012;2012:383829.
8. Faridgohar M, Nikoueinejad H. New findings of Toll-like receptors involved in Mycobacterium tuberculosis infection. Pathog Glob Health. 2017;111(5):256-64.
9. Liu Y, Wang Y, Yamakuchi M, Isowaki S, Nagata E, Kanmura Y, et al. Upregulation of toll-like receptor 2 gene expression in macrophage response to peptidoglycan and high concentration of lipopolysaccharide is involved in NF-kappa b activation. Infect Immun. 2001;69(5):2788-96.
10. Lin PL, Plessner HL, Voitenok NN, Flynn JL. Tumor necrosis factor and tuberculosis. J Investig Dermatol Symp Proc. 2007;12(1):22-5.

11. Flo TH, Halaas O, Torp S, Ryan L, Lien E, Dybdahl B, et al. Differential expression of Toll-like receptor 2 in human cells. J Leukoc Biol. 2001;69(3):474-81.
12. Huang Z-F, Massey JB, Via DP. Differential regulation of cyclooxygenase-2 (COX-2) mRNA stability by interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) in human in vitro differentiated macrophages. Biochem Pharmacol. 2000;59(2):187-94.
13. Wu MY, Lu JH. Autophagy and Macrophage Functions: Inflammatory Response and Phagocytosis. Cells. 2019;9(1).
14. Chin MS, Nagineni CN, Hooper LC, Detrick B, Hooks JJ. Cyclooxygenase-2 gene expression and regulation in human retinal pigment epithelial cells. Invest Ophthalmol Vis Sci. 2001;42(10):2338-46.
15. Kang YJ, Mbonye UR, DeLong CJ, Wada M, Smith WL. Regulation of intracellular cyclooxygenase levels by gene transcription and protein degradation. Prog Lipid Res. 2007;46(2):108-25.