Impaired expression of the TWIK2 K+ efflux channel leads to reduced IL-1 family cytokine production in patients with nontuberculous mycobacterial lung disease

Bock-Gie Jung (✉ bockgie.jung@uthct.edu)  
The University of Texas Health Science Center at Tyler

Kristin Dean  
The University of Texas Health Science Center at Tyler

Carly Wadle  
The University of Texas Health Science Center at Tyler

Buka Samten  
The University of Texas Health Science Center at Tyler

Deepak Tripathi  
The University of Texas Health Science Center at Tyler

Richard J. Wallace, Jr.  
The University of Texas Health Science Center at Tyler

Barbara A. Brown-Elliott  
The University of Texas Health Science Center at Tyler

Tony Tucker  
The University of Texas Health Science Center at Tyler

Steven Idell  
The University of Texas Health Science Center at Tyler

Julie V. Philley  
The University of Texas Health Science Center at Tyler

Ramakrishna Vankayalapati  
The University of Texas Health Science Center at Tyler

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Abstract

**Background:** Nontuberculous mycobacteria (NTM) causes disseminated disease in patients with immunodeficiency and pulmonary disease in individuals without obvious immunodeficiency. There is no clear information on how NTM pulmonary disease (NTMPD) develops in immunocompetent hosts. This profile study was initiated to gain insight into the immunological factors that predispose persons to pulmonary NTM infections.

**Methods:** Blood samples were obtained from 15 pairs of NTMPD patients and age-matched healthy household contacts. Peripheral blood mononuclear cells (PBMCs) were stimulated with the heat-killed *M. avium* complex (MAC). A total of 34 cytokines and chemokines were evaluated in PBMCs culture supernatants and plasma using multiplex immunoassays. The mRNA expression of TLR2, P2X7R, TWIK2, THIK2 and TREK1 was measured by quantitative real-time PCR. In mechanistic studies, blood samples were obtained from healthy volunteers who had not been diagnosed or treated for NTM.

**Results:** Interleukin (IL)-1β, IL-18, IL-1α and IL-10 production were significantly reduced in response to MAC in PBMCs of NTMPD patients compared with PBMCs of their healthy household contacts. RANTES was the only chemokine significantly elevated in the plasma of NTMPD patients compared with plasma of their healthy household contacts. TLR2 and TWIK2 expression were impaired in response to MAC in PBMCs of NTMPD patients compared with PBMCs of their healthy household contacts. RANTES had no effect on IL-1β production by macrophages infected with MAC. A TLR2 inhibitor decreased IL-1β, IL-18, IL-1α and IL-10 production by MAC-stimulated PBMCs and monocytes. A TWIK2 inhibitor decreased the production of IL-1β, IL-18, and IL-1α, but not IL-10, by MAC-stimulated PBMCs and monocytes.

**Conclusions:** These findings suggest that impaired TWIK2 results in decreased production of IL-1 family cytokines (IL-1β, IL-18, and IL-1α) in response to MAC, consequentially may increase susceptibility to NTM pulmonary infection.

Introduction

Nontuberculous mycobacteria (NTM) refer to all members of the genus *Mycobacterium* except for the *M. tuberculosis* complex, which causes tuberculosis (TB), and *M. leprae* and *M. lepromatosis*, which cause leprosy (1–3). NTM can cause disseminated disease in patients with immunodeficiencies, whether primary or acquired, but these organisms mainly cause isolated pulmonary disease in individuals without obvious immunodeficiency (3, 4). *M. avium* complex (MAC), which is mainly consists of *M. avium* and *M. intracellulare*, is the most frequently isolated group in patients with NTM pulmonary diseases (NTMPD) (3).

Recently, the incidence and prevalence of NTMPD have increased worldwide. In the United States from 2008 to 2015, the annual incidence of NTMPD increased from 3.13 to 4.73 per 100,000 person/years, and the annual prevalence increased from 6.78 to 11.70 per 100,000 persons (5). These trends are consistent with other studies from Europe (6), South Korea (7), and Japan (8). Although immunological risk factors...
that cause NTM disseminated infection have been well-studied in immunocompromised hosts, there is no clear information on how NTMPD develops in immunocompetent hosts.

NTM disseminated infection is associated with a reduction in CD4+ T cells in patients with acquired immunodeficiency syndrome (AIDS) (9) and a deficiency in genes affecting the interleukin (IL)-12 and interferon (IFN)-γ pathways in patients with Mendelian susceptibility to mycobacterial disease (MSMD) (4). However, there is no evidence of a reduction in CD4+ T cells in patients with NTMPD (10). IL-12 and IFN-γ responses in patients with NTMPD have been inconsistent, with reports of increased (11), unchanged (10) or decreased (12–14) levels. Similar inconsistencies have been reported regarding the role of tumor necrosis factor (TNF)-α in patients with NTMPD. Some studies have reported attenuated TNF-α responses (12, 14, 15), but others found an intact TNF-α response (10, 13) in patients with NTMPD.

To gain insight into the immunologic factors that contribute to the development of NTMPD, we evaluated the immune responses of NTMPD patients and their healthy household contacts. We found defective IL-1β, IL-18, IL-1α and IL-10 production by patient peripheral blood mononuclear cells (PBMCs) in response to MAC antigens. We also determined the mechanisms responsible for defective production of these cytokines.

**Methods**

**Subjects**

Blood samples were obtained from 15 patients with a diagnosis of NTMPD at the University of Texas Health Science Center at Tyler from July 2019 to November 2019. A diagnosis was made on the basis of guidelines recommended by the American Thoracic Society (1). In all cases, disease was confined to the lungs. None of the patients had risk factors for human immunodeficiency virus (HIV) infection. All patients were Caucasian females with a median age of 65 years (range, 48-83 years). Healthy household contacts were used as an age-matched healthy control [a median age of 68 years (range, 57-82 years)]. In mechanistic studies, blood samples were obtained from healthy volunteers who had not been diagnosed or treated for NTM. All studies were reviewed and approved by the Institutional Review Board of the University of Texas Health Science Center at Tyler (protocol #1085), and written informed consent was obtained from all study subjects.

**Bacterial source and stock**

*M. intracellulare* (clinical isolate) and the *M. avium* Chester (ATCC 700898) were provided by the Department of Microbiology, the University of Texas Health Science Center at Tyler, Texas. The bacteria were cultured and prepared as described in our previous study (16).

**Heat-killed MAC antigens**

*M. intracellulare* and *M. avium* were heated at 100 °C for 5 min for inactivation as previously described (17). The bacteria were sonicated on ice, and the crude lysates were used as heat-killed MAC antigens.
The protein concentration of the MAC antigens was measured using a commercial BCA protein assay kit. Heat-killed MAC antigens were resuspended in PBS (5 mg/ml), aliquoted and stored at -80 °C until use.

**PBMC stimulation with heat-killed MAC antigens**

To compare immune responses between NTMPD patients and their own healthy household contacts, PBMCs were isolated from whole blood using density gradient centrifugation with Ficoll-Paque (GE Healthcare). Isolated PBMCs were resuspended at a density of 2 × 10^6 cells/ml in RPMI-1640 supplemented with 10% heat-inactivated human serum. The cells were then placed in 12-well plates and incubated in the presence or absence of 10 μg/ml heat-killed MAC antigens at 37 °C in 5% CO₂. After a 72 h incubation, the cell-free supernatants were collected, and the cell pellets were treated with TRlzol LS reagent (Invitrogen) and stored at -80 °C for subsequent multiplex immunoassay and quantitative real-time PCR, respectively.

**Plasma**

Peripheral blood samples were collected into tubes containing sodium heparin and centrifuged at 10,000 × g for 10 min at 4 °C within 30 min of collection. Plasma samples were collected and stored at -80 °C until use.

**Multiplex immunoassay**

A total of 34 cytokines and chemokines were evaluated in the culture supernatants of PBMCs and the plasma from NTMPD patients and their own healthy household contacts using commercial multiplex immunoassay kits (34-Plex Human ProcartaPlex Panel 1A, Invitrogen) according to the manufacturer’s instructions.

**Macrophage stimulation with live M. intracellulare**

Monocyte-derived macrophages (MDM) of healthy volunteers were prepared (18) and infected with live *M. intracellulare* at a multiplicity of infection (MOI) of 20 as previously described (19). The cells were further incubated in the presence of various concentrations (0, 100 or 300 ng/ml) of recombinant RANTES (R&D Systems) or plasma from NTMPD patients (10 μl) at 37 °C in 5% CO₂. In some cases, the plasma was pretreated with various concentrations (0, 0.5 or 1 μg/ml) of anti-RANTES antibody (R&D Systems) or 1 μg/ml of isotype control antibody (R&D Systems) at 37 °C for 30 min. After 24 h of incubation, cell culture supernatants were collected and stored at -80 °C for subsequent cytokine measurements.

**Quantitative real-time PCR**

The mRNA expression of toll-like receptor (TLR) 2, P2X7 receptor (P2X7R), *Kcnk2* (encoding TREK1), *Kcnk6* (encoding TWIK2) and *Kcnk12* (encoding THIK2) was measured by real-time PCR using 18S as an internal control with specific primer and probe sets (Additional file 4: Table S1, Applied Biosystems).
**Pharmacological inhibition of TLR2 and TWIK2**

C29 (MedChemExpress) and quinine (Sigma) were used as TLR2-selective inhibitors (20) and TWIK2-selective inhibitors (21, 22), respectively. PBMCs from healthy volunteers were stimulated with 10 µg/ml heat-killed MAC antigens in the presence or absence of various concentrations (10, 50 and 100 µM) of C29 or quinine at 37 °C in 5% CO$_2$ for 24 h. CD14+ monocytes from healthy volunteers were stimulated with live MAC (MOI of 10) in the presence or absence of various concentrations (10, 50 and 100 µM) of C29 or quinine at 37 °C in 5% CO$_2$ for 18 h. The cell-free supernatants were collected and stored at -80 °C until use.

**ELISA and LDH assay**

For the experiment of pharmacological inhibition of TLR2 and TWIK2, commercial ELISA kits were used to determine the amounts of human IL-1β (Mabtech), human IL-18 (R&D Systems), human IL-1α (Biolegend) and human IL-10 (Biolegend) in the culture supernatants of PBMCs and CD14+ monocytes. A colorimetric LDH assay kit (Abcam) was used to determine the LDH activity in culture supernatants of PBMCs and CD14+ monocytes.

**Statistical analysis**

The results are expressed as the means ± SEM. In the case of comparisons between NTMPD patients and their own healthy household contacts, a paired t test was performed. For other comparisons, an unpaired t test (two-tailed) was performed. $P < 0.05$ was considered significant.

**Results**

**Cytokine and chemokine production by PBMCs of NTMPD patients and healthy household contacts in response to MAC antigens**

We cultured PBMCs from 15 NTMPD patients and 15 healthy household contacts with heat-killed MAC antigens as mentioned in the methods section. After 72 h, culture supernatants were collected, and 34 cytokine and chemokine levels were measured by multiplex immunoassay. PBMCs from NTMPD patients produced significantly less IL-1β, IL-18, IL-1α and IL-10 than PBMCs from their healthy household contacts in response to heat-killed MAC antigens (Fig. 1A-D). There were no significant differences in other 30 cytokines and chemokines production by MAC-stimulated PBMCs between NTMPD patients and healthy household contacts (Additional file 1: Fig. S1). In response to heat-killed *M. intracellulare* antigen, PBMCs of NTMPD patients produced $4187.0 ± 681.1$ pg/ml IL-1β, $46.9 ± 4.3$ pg/ml IL-18, $52.4 ± 7.0$ pg/ml IL-1α, and $1178.0 ± 167.9$ pg/ml IL-10, whereas PBMCs of their healthy household contacts produced $6819.0 ± 895.7$ pg/ml IL-1β ($P < 0.01$, Fig. 1A), $76.0 ± 11.5$ pg/ml IL-18 ($P < 0.05$, Fig. 1B), $67.2 ± 8.9$ pg/ml IL-1α ($P < 0.01$, Fig. 1C), and $1715.0 ± 242.0$ pg/ml IL-10 ($P < 0.01$, Fig. 1D). Similar trends were noted in response to heat-killed *M. avium* antigen (Fig. 1). Our findings demonstrate that PBMCs of
NTMPD patients produce less IL-1β, IL-18, IL-1α and IL-10 in response to MAC antigens than PBMCs of their healthy household contacts.

**Cytokine and chemokine levels in the plasma of patients and healthy household contacts**

We also measured cytokine and chemokine levels by multiplex immunoassay in the plasma of patients and healthy household contacts. Among 34 cytokines and chemokines, RANTES levels were significantly higher in NTMPD patient plasma (22.6 ± 1.2 pg/ml) than in healthy household contact plasma (15.5 ± 1.2 pg/ml) \( (P < 0.001; \text{Fig. 2A}) \). There was no significant difference in other 33 plasma cytokines and chemokines between NTMPD patients and their healthy household contacts (Additional file 2: Fig. S2).

**Effect of RANTES on IL-1β production by macrophages**

The essential role of IL-1β in protection against mycobacterial diseases has been well demonstrated (23-25). Since RANTES was the only chemokine significantly elevated in the plasma of patients, we determined whether RANTES can affect IL-1β production by macrophages. Monocyte-derived macrophages (MDM) were infected with *M. intracellulare* as mentioned in the methods section at an MOI of 20 and incubated in the presence of various concentrations of recombinant RANTES or plasma from NTMPD patients (in some cases, plasma was pretreated with an anti-RANTES antibody or isotype antibody as mentioned in the methods section) at 37 °C in 5% CO₂. Cell culture supernatants were collected after 24 h of incubation, and IL-1β concentrations were measured by ELISA (Fig. 2B). *M. intracellulare*-induced IL-1β (397.1 ± 130.8 pg/ml) was not significantly changed by recombinant RANTES (443.0 ± 155.8 pg/ml at 100 ng/ml RANTES, 440.5 ± 138.4 pg/ml at 300 ng/ml RANTES) or plasma from NTMPD patients (559.0 ± 82.5 pg/ml after addition of plasma, 559.0 ± 82.5 pg/ml after addition of plasma pretreated with 0.5 ng/ml anti-RANTES antibody, 492.3 ± 94.0 pg/ml after addition of plasma pretreated with 1 ng/ml anti-RANTES antibody, 524.3 ± 112.9 pg/ml after addition of plasma pretreated with 1 ng/ml anti-RANTES antibody). These data suggest that RANTES has no effect on IL-1β production by macrophages infected with *M. intracellulare*.

**NTMPD patients have a defective expression of TLR2 and TWIK2**

TLR2, P2X7R and two-pore domain potassium (K+) efflux channels are involved in mycobacteria-induced proinflammatory signaling, especially IL-1β production (22, 26, 27). To determine whether any of these receptors and channels are involved in reduced IL-1β, IL-18, IL-1α and IL-10 production, we cultured PBMCs of NTMPD patients and their healthy household contacts with or without heat-killed *M. intracellulare* as mentioned in the methods section. We determined the relative mRNA expression levels of TLR2, P2X7R and two-pore domain K+ efflux channels, including TWIK2, THIK2 and TREK1 (Fig. 3). Upon stimulation with heat-killed *M. intracellulare* antigen, the relative mRNA expression of TLR2 and TWIK2 in PBMCs was significantly reduced in NTMPD patients compared to their healthy household contacts (Fig. 3A and 3C). There was no difference in the expression of P2X7R and THIK2 in these PBMCs. (Fig. 3B and 3D). Of note, the relative mRNA expression level of TWIK2 in PBMCs was significantly reduced in NTMPD patients compared to their healthy household contacts even without stimulation (Fig. 3C). TREK1
expression was not detected in any samples until 40 cycles of real-time PCR amplification (data not shown). These data suggest that the expression of TLR2 and TWIK2 is impaired in NTMPD patients.

**TLR2 inhibition reduces IL-1β, IL-18, IL-1α and IL-10 production by PBMCs and monocytes in response to MAC.**

We next determined whether reduced expression of TLR2 is related to reduced production of IL-1β, IL-18, IL-1α and IL-10 by PBMCs stimulated with heat-killed MAC antigens. PBMCs from 4 healthy donors were cultured with or without heat-killed MAC antigens in the presence or absence of a TLR2-selective inhibitor (C29). The TLR2-selective inhibitor significantly reduced IL-1β, IL-18, IL-1α and IL-10 production by heat-killed MAC antigen-stimulated PBMCs in a dose-dependent manner (Fig. 4A-4D). Monocytes and macrophages are known as the major sources of IL-1β, IL-18 and IL-1α. We next determined whether TLR2 is involved in the production of IL-1β, IL-18, IL-1α and IL-10 by monocytes infected with live MAC. Monocytes from 6 healthy donors were infected with or without MAC in the presence or absence of a TLR2-selective inhibitor (C29). The TLR2-selective inhibitor significantly reduced the production of IL-1β, IL-18, IL-1α and IL-10 by MAC-infected CD14+ monocytes in a dose-dependent manner (Fig. 4E-4H). The TLR2-selective inhibitor (C29) had no effect on cell viability (Additional file 3: Fig. S3A and S3B). These data suggest that TLR2 is involved in IL-1β, IL-18, IL-1α and IL-10 production by PBMCs and monocytes in response to MAC.

**TWIK2 inhibition reduces IL-1β, IL-18 and IL-1α, but not IL-10, production by PBMCs and monocytes in response to MAC.**

We next determined whether the reduced expression of TWIK2 is related to the reduced production of IL-1β, IL-18, IL-1α and IL-10 by PBMCs stimulated with heat-killed MAC antigens. PBMCs from 4 healthy donors were cultured with or without heat-killed MAC antigens in the presence or absence of a TWIK2-selective inhibitor (quinine). The TWIK2-selective inhibitor significantly reduced IL-1β, IL-18 and IL-1α, but not IL-10, production by heat-killed MAC antigen-stimulated PBMCs in a dose-dependent manner (Fig. 5A-5D). We next determined whether TWIK2 is involved in the production of IL-1β, IL-18, IL-1α and IL-10 by monocytes infected with live MAC. Monocytes from 7 healthy donors were infected with or without MAC in the presence or absence of a TWIK2-selective inhibitor (quinine). The TWIK2-selective inhibitor significantly reduced IL-1β, IL-18 and IL-1α, but not IL-10, production by MAC-infected CD14+ monocytes in a dose-dependent manner (Fig. 5E-5H). The TWIK2-selective inhibitor (quinine) had no effect on cell viability (Additional file 3: Figs. S3C and S3D). These data suggest that TWIK2 is involved in IL-1β, IL-18 and IL-1α, but not IL-10, production by PBMCs and monocytes in response to MAC.

**Discussion**

NTM causes disseminated disease in patients with immunodeficiency and its immunological risk factors have been well-established (1, 4). Whereas limited information is available on the nature of immune dysfunction in NTM patients with pulmonary diseases (3). In the current study, we compared the immune responses of NTMPD patients and their healthy household contacts in response to MAC antigens. Our
findings demonstrate that in response to MAC antigens, PBMCs from NTMPD patients produced less IL-1β, IL-18, IL-1α and IL-10 and that TLR2 and TWIK2 expression was reduced compared to their healthy household contacts. We also found that inhibition of TLR2 decreased the production of IL-1β, IL-18, IL-1α and IL-10 by PBMCs and monocytes from healthy donors in response to MAC, whereas inhibition of TWIK2 decreased the production of IL-1β, IL-18 and IL-1α, but not IL-10, by PBMCs and monocytes from healthy donors in response to MAC.

The essential role of IL-12, IFN-γ and TNF-α in the host defense against TB (28, 29) and NTM disseminated disease (3, 4) is well documented. However, there are conflicting reports about their role in NTMPD (10–15). In the current study, we found that PBMCs from NTMPD patients and their healthy household contacts produced similar amounts of IL-12, IFN-γ and TNF-α in response to MAC antigens (Additional file 1: Fig. S1). IL-1 family cytokines (IL-1β, IL-18 and IL-1α) produced by antigen-presenting cells are known to play an important role against various pathogens, including *M. tuberculosis* (23–25). Similarly, PBMCs and macrophages from NTMPD patients produce less IL-1β and IL-18 than those from healthy donors in response to MAC antigen (30) or LPS (10). However, the factors responsible for the reduced IL-1β levels in PBMCs from NTMPD patients are unknown. Our current results confirm the above findings and further identify a possible mechanism for the reduced production of IL-1β, IL-18 and IL-1α by PBMCs and monocytes of NTMPD patients.

Plasma RANTES levels were significantly higher in NTMPD patients than in the plasma of their healthy household contacts, similar to previous findings in *M. abscessus*- and *M. tuberculosis*-infected patients (31, 32). RANTES is a potent chemoattractant factor for monocytes and T cells and is associated with the function of cytotoxic T cells, which are important in the control of various intracellular pathogens (33). RANTES polymorphisms are associated with an increased risk of TB (34–36). In the current study, we found that recombinant RANTES protein or plasma RANTES did not significantly alter IL-1β production by macrophages (Fig. 2B), suggesting that increased levels of RNATES are not responsible for the attenuated IL-1 responses in NTMPD patients.

IL-10 is a potent anti-inflammatory cytokine and is well known as a deleterious cytokine during infection with various pathogens mainly via deactivation of macrophages, resulting in diminished Th1 cytokine production (37). There are conflicting reports on IL-10 production by PBMCs of NTMPD patients in response to MAC antigens. PBMCs from NTMPD patients produce more IL-10 than PBMCs from healthy donors in response to MAC antigens (heat-killed *M. intracellulare*) (12), tuberculin and sensitin (11); other studies found no significant difference between NTMPD patients and healthy subjects (10, 14). Another study found that whole blood from NTMPD patients produced less IL-10 than healthy donors in response to LPS, heat-killed *Staphylococcus epidermidis*, or live *M. intracellulare* (13). In addition to IL-1β, IL-18 and IL-1α, PBMCs from NTMPD patients produced less IL-10 in response to MAC antigens (Fig. 1D).

A two-step mechanism is required for the production and release of IL-1 family cytokines such as IL-1β by macrophages (38). First, transcription and translation of the inactive form of IL-1β are initiated from recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs),
such as TLRs. Second, assembly of the inflammasome is induced by further signals such as K+ efflux. This assembled inflammasome complex induces the maturation and release of IL-1β (39). TLRs, especially TLR2, play an important role in the innate host response to mycobacteria, leading to the production of cytokines (26). P2X7R plays a central role in the maturation and secretion of IL-1β by activating ATP-dependent K+ efflux (39). A recent study suggested that TWIK2 (a member of the two-pore domain K+ efflux channel family) activates P2X7R independent-K+ efflux and mediates the production of IL-1β in macrophages (21). We found reduced expression of TLR2 and TWIK2, but not P2X7R, THIK2 or TREK1, in PBMCs of NTMPD patients compared to their healthy household contacts. Inhibition of TLR2 expression on PBMCs and monocytes of healthy donors at the time of MAC antigen stimulation or MAC infection reduced IL-1β, IL-18, IL-1α and IL-10 production, whereas inhibition of TWIK2 expression on PBMCs and monocytes of healthy donors at the time of MAC antigen stimulation or MAC infection reduced IL-1β, IL-18 and IL-1α, but not IL-10, production. Our findings suggest that impaired expression of TLR2 can lead to defective production of IL-1β, IL-18, IL-1α and IL-10 in NTMPD patients. However, impaired expression of TWIK2 is responsible for defective production of IL-1β, IL-18 and IL-1α, but not IL-10, in NTMPD patients.

A previous study showed that TLR2 gene polymorphisms are associated with the development of NTMPD (30, 40). The TWIK2 K+ efflux channel mediates NLRP3 inflammasome-induced inflammation in macrophages (21), and an attenuated NLRP3 inflammasome is correlated with decreased IL-1β responses and host susceptibility in NTMPD patients (30). However, activation of the NLRP3 inflammasome by M. tuberculosis is uncoupled from susceptibility to active TB (41). These findings may suggest that TWIK2-mediated NLRP3 inflammasome activation is involved in host defense against NTMPD but not TB. Therefore, future studies to understand the precise mechanisms associated with TWIK2-mediated NLRP3 inflammasome activation in NTMPD are needed to develop immunomodulatory strategies to treat or prevent NTMPD.

There were some limitations to this study. To avoid interruption by environmental risk factors, we used each patient’s own healthy household contacts as healthy controls. However, there was a difference in gender between NTMPD patients (15 female) and healthy household contacts (1 female, 14 male). Thus, data may be affected by a difference in gender. The subject number was small, and some significance may have been underestimated. All patients and healthy household contacts were Caucasian. Thus, the results may not be generalized to other ethnicities.

In the current study, we found that defective expression of TLR2 and TWIK2 in NTMPD patients leads to defective cytokine production, which is known to control bacterial growth. Since IL-1 signaling, especially IL-1β, is essential for host defense against mycobacterial infection, it is important to further understand whether defective TLR2 and/or TWIK2 expression or gene polymorphisms cause susceptibility to NTMPD infection or contribute to the development of NTMPD.

**Declarations**
Ethics approval and consent to participate: All studies were reviewed and approved by the Institutional Review Board of the University of Texas Health Science Center at Tyler (protocol #1085). Informed consent was obtained from all individual participants included in the study.

Consent for publication: Informed consent was obtained from all individual participants included in the study.

Availability of data and materials: All data generated in this study are provided in the main manuscript and supplemental information.

Competing interests: The authors have no relevant financial or non-financial interests to disclose.

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Authors' contributions: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by BJ, KD and CW. The first draft of the manuscript was written by BJ and RV, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

References


Figures

Figure 1

Production of cytokines and chemokines by M. avium complex (MAC)-stimulated peripheral blood mononuclear cells (PBMCs). PBMCs were isolated from 15 pairs of NTMPD patients (NTM) and healthy household contacts (HHC) and cultured in the presence or absence of 10 μg/ml heat-killed MAC [M. intracellulare (M.i) or M. avium (M.a)] for 72 h. The concentrations of 34 cytokines and chemokines were measured by multiplex immunoassay. Data for cytokines interleukin (IL)-1β (A), IL-18 (B), IL-1α (C), and IL-10 (D) are shown. Data are expressed as the means ± SEM. *P < 0.05, and **P < 0.01.

Figure 2

Plasma RANTES levels (A) and their effect on IL-1β production by macrophages (B). Plasma samples were collected from 15 pairs of NTMPD patients (NTM) and their healthy household contacts (HHC). Concentrations of 34 cytokines and chemokines were measured by multiplex immunoassay. Monocyte-derived macrophages (MDM) from 3 healthy volunteers were infected with M. intracellulare at a multiplicity of infection (MOI) of 20 in the presence or absence of RANTES (100 or 300 ng/ml) or plasma from NTMPD patients [10 μl of plasma were pretreated with or without anti-RANTES antibody (0.5 or 1 μg/ml) or isotype antibody] for 24 h. The concentration of IL-1β was measured by ELISA. Data are expressed as the means ± SEM. ***P < 0.001. ns, not significant.
TLR2 (A), P2X7 receptor (B), TWIK2 (C), and THIK2 (D) expression in *M. intracellulare*-stimulated PBMCs. PBMCs were isolated from 15 pairs of NTMPD patients (NTM) and healthy household contacts (HHC), and the cells were cultured in the presence or absence of 10 μg/ml heat-killed *M. intracellulare* for 72 h. Gene expression was measured by real-time PCR using 18S as an internal control with specific primer and probe sets. TREK1 signals were not detected in any samples until 40 cycles of real-time PCR. Data are expressed as the means ± SEM. *P < 0.05, **P < 0.01, and ***P < 0.001. ns, not significant.

**Figure 4**

Effect of a TLR2 inhibitor on the production of IL-1β, IL-18, IL-1α, and IL-10 by PBMCs and CD14+ monocytes stimulated with *M. avium* complex (MAC). PBMCs from 4 healthy volunteers were stimulated with 10 μg/ml heat-killed MAC antigens in the presence or absence of various concentrations (10, 50 and 100 μM) of C29 (a TLR2-selective inhibitor) for 24 h (A-D). Monocytes of 6 healthy volunteers were infected with live MAC at an MOI of 10 in the presence or absence of various concentrations (10, 50 and 100 μM) of C29 (a TLR2-selective inhibitor) for 18 h (E-H). The concentrations of cytokines were measured by ELISA. Data are expressed as the means ± SEM. *P < 0.05, **P < 0.01, and ***P < 0.001. ns, not significant.

**Figure 5**

Effect of a TWIK2 inhibitor on the production of IL-1β, IL-18, IL-1α, and IL-10 by PBMCs and CD14+ monocytes stimulated with *M. avium* complex (MAC). PBMCs from 4 healthy volunteers were stimulated with 10 μg/ml heat-killed MAC antigens in the presence or absence of various concentrations (10, 50 and 100 μM) of quinine (a TWIK2-selective inhibitor) for 24 h (A-D). Monocytes of 7 healthy volunteers were infected with live MAC at an MOI of 10 in the presence or absence of various concentrations (10, 50 and 100 μM) of quinine (a TWIK2-selective inhibitor) for 18 h (E-H). The concentrations of cytokines were measured by ELISA. Data are expressed as the means ± SEM. *P < 0.05, **P < 0.01, and ***P < 0.001. ns, not significant.

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