European Review for Medical and Pharmacological Sciences 2018; 22: 4228-4237

PKC δ gene can induce macrophages to release inflammatory factors against *Mycobacterium tuberculosis* infection

Y.-T. DUAN[1], K.-Y. BI[1], Y.-S. MA[2]

[1]Department of Tuberculosis, Liaocheng Infectious Disease Hospital, Liaocheng, China
[2]Department of Health, The People Hospital of Weifang, Weifang, China

Abstract. – OBJECTIVE: To investigate the effect of PKC δ gene on the anti-tuberculosis activity of macrophages and the mechanism.

MATERIALS AND METHODS: Bone marrow cells of PKC δ knockout mice and wild-type mice were cultured and L929 cells were induced to differentiate into macrophages. Lipopolysaccharide (LPS) and trehalose 6,6'-dimycolate (TDM) were used to stimulate macrophages respectively. After 24 and 96 hours, cells and the supernatant were collected to evaluate the inflammatory cytokines produced by macrophages using ELISA method. Real-time PCR was performed to detect the expression of macrophage mRNA level and nitric oxide (NO) production of macrophages was measured by NO assay.

RESULTS: The results showed that, after TDB stimulation, IL-1β, IL-6, and other cytokines, as well as NO produced by macrophages of PKC δ knockout mice, were significantly decreased ($p < 0.01$) compared with the wild-type mice. In PKC δ knockout macrophages, the above protein-coding genes were also decreased significantly at the transcriptional level ($p < 0.01$).

CONCLUSIONS: PKC δ can enhance the anti-tuberculosis capacity of macrophages by inducing to the release of inflammatory factors by macrophages.

Key Words:
Tuberculosis, Macrophage, PKC, Inflammatory factor.

Introduction

Pulmonary tuberculosis is one of the three major infectious diseases that threaten human health, and the second most fatal infectious disease that is second only to AIDS. The latest statistics from WHO showed that 1/3 of the global population, about 2 billion people, were currently infected. On average, more than 2 million people die of tuberculosis each year, with the death population concentrating mainly in developing countries such as China[1].

As the first barrier of the immune system, the innate immune system mediated by neutrophils and macrophages plays an important role in the pathological process of infection[2]. Macrophages are the main antigen-presenting cells of *Mycobacterium tuberculosis* (MTB) antigen. MTB was identified for phagocytosis by macrophages, then encapsulated by the plasma membrane and finally formed lysosomes. It was degraded and processed by Cathepsin (Cath), making the MTB antigen some small peptide segments with immunogenicity. Some of the peptide segments can be transported to the surface of macrophages to activate CD4+ T cells after binding with appropriate MHC class II molecules. In addition, the activated CD4+ T cells can secrete TNF-α, IFN-γ, IL-12 to further enhance the function of macrophages[3-5].

PKC δ is a member of the Ca^{2+} insensitive protein kinase C family. In resting cells, it is mainly distributed in the cytoplasm presenting a non-activate conformation. Once diacylglycerol (DAG) and phosphatidylserine (PS) exist, PKC δ will become a membrane-bound enzyme to activate enzymes in the cytoplasm, thereby involving in the regulation of a variety of biochemical reactions. At the same time, it can act on the transcription factors in the nucleus to participate in the regulation of gene expression. Therefore, PKC δ is actually a multifunctional enzyme[6]. Protein kinase C is a multifunctional serine and threonine kinase. At present, at least 15 kinds of protein kinase C subtypes have been found, and PKC δ was found to be the key protein for the normal function of innate immune cells[7]. Waki et

Corresponding Author: Yongsheng Ma, MM; e-mail: 313921192@qq.com

al[8] found that compared to other PKC subtypes, only PKC δ can affect the phagocytosis of neutrophils as well as regulate the activity of NADPH oxidase. Bey et al[9] used inhibitors of different PKC subtypes to treat cells, and it was found that only PKC δ participated in the phosphorylation and cytoplasm-nuclei transportation of p47phox subunit, which is a NADPH oxidase of human monocyte.

In this study, a PKC δ knockout mouse model was used. After stimulation of macrophages with trehalose 6,6-dibehenate (TDB) *in vitro*, changes of cytokines and NO secretion production by macrophages in wild-type and mutant mice as well as RNA transcription levels of the above inflammatory mediators were compared. The study of the role of PKC δ in macrophages against *Mycobacterium tuberculosis* infection is of great significance for exploring the specific molecular mechanisms of anti- *Mycobacterium tuberculosis* infection. Meanwhile, it also provides a theoretical basis for tuberculosis prevention and immunotherapy.

Materials and Methods

Animal and Cell Culture

PKC δ knockout mice (PKC δ-/-) were established with wild-type C57BL/6 mice as control. The 6-7-week-old mice were sacrificed by CO_2 asphyxia and placed in 75% alcohol for 2-3 minutes. The mice were placed on the KIMTECH paper with the whole leg bones cut off. The leg bone was put in a sterile Petri dish with joints cut. 5 mL Dulbecco's Modified Eagle Medium (DMEM) complete medium was taken into the syringe, the syringe needle was inserted into the marrow cavity for flushing. 15 mL centrifuge tube and cell filter were taken for cell collecting, and the cell suspension was added to the filter screen. Then, the suspension was collected into 15 mL centrifuge tube. The bone was cut into pieces and 2 mL DMEM was added to wash the culture dish and filter screen. Then the suspension was centrifuged for 3 minutes at room temperature, with the supernatant discarded. 1ml erythrocyte lysis buffer was added to the tube, then the red cell was completely cracked and 5 mL DMEM complete culture medium was added to the lysate. Once more, the lysate was added to the filter screen for removing the red cell membrane. The cells were centrifuged for 3 minutes at room temperature, and the supernatant was abandoned. The precipitate of cells was the stem cells of the bone marrow.

L929 cells can secrete macrophage colony stimulating factor (M-CSF), which can induce bone marrow cells to differentiate into macrophages. In the process of L929 cell culture, cells released this cytokine into the supernatant of the culture medium. Therefore, we collected M-CSF from the supernatant after 5-7 days of L929 cell culture. The collected supernatant was first centrifuged at low speed for impurity removing, then filtrated with a filter equipped with 0.22 μM filter membrane, and stored at -20°C. This study was approved by the Animal Ethics Committee of The People Hospital of Weifang Animal Center.

An appropriate amount of DMEM medium was added, and the extracted bone marrow cells were seeded into the cell culture plate. At the same time, 30% L929 supernatant and 5% fetal bovine serum (FBS) were added to the plate, which was incubated in 37°C 5% CO_2 concentration saturated humidity environment. The bone marrow hematopoietic stem cells were induced and developed into mature primary macrophages under the mediate of M-CSF in the L929 supernatant for 7 days. The purity of macrophages was identified by flow cytometry.

Macrophages were stimulated with 100 ng/mL LPS and 20 μg/mL TDB respectively. After stimulated for 24 hours and 96 hours, the cells and cell supernatant were collected for examination.

Mouse Genotyping by Polymerase Chain Reaction (PCR)

About 5 mm mouse tail was cut and put into a 1.5 mL centrifuge tube, and 100 μL 25 mM NaOH was added in the tube. The tube was put into the boiling water for 1 hour. Subsequently, the tail was shaked, mashed and kept in boiling water for 30 minutes to make sure it was completely digested. 100 μL 40 mM Tri-HCl (pH 5.4) was added to neutralize NaOH and mixed upside down. The tube was then centrifuged at room temperature for 10 minutes. The mouse genome DNA in the supernatant was used as a template to detect the mouse genotypes by PCR. The results of genotyping were observed in the ultraviolet transmission reflectance analyzer after electrophoresis. With the comparison of DNA marker, the size of the target DNA fragment was semi quantitatively analyzed. PKC δ primers

for genotype identification: Primer 1: 5'-GACCGCAGCTGAAGTCAGTGTTTC-3' Primer 2: 5'-CCAGCAGGTCATGAACTTGTAAC-3'

Detection of the Expression of Il-1β, Il-6 and iNOS mRNA in Cells by Fluorescence Quantitative PCR

A proper amount of chloroform (chloroform: TRIzol 1:5) was dissolved in cell samples treated with TRIzol and mixed at room temperature for 5 minutes. After centrifugation at 4°C for 15 minutes, the supernatant was transferred to a new 1.5 mL centrifuge tube, then isopropanol was added to the tube and mixed at room temperature for 5-10 minutes. After centrifugation at 4°C for 15 minutes, the supernatant was abandoned and 1 mL 70% Ethanol (DEPC water) was added to the white precipitate for cleaning. Then the tube was centrifuged for 15 minutes. The supernatant was abandoned and the precipitated RNA was dried at room temperature for several minutes. At last, the concentration of RNA was detected.

PCR programs were as follows, 95°C pre-denaturation for 3 minutes, 95°C denaturation for 15 seconds, 58°C annealing for 20 seconds, 72°C extension for 15 seconds, with 40 cycles. After the end of Real-Time PCR reaction, the expression level of mRNA could be calculated according to the parameters of amplification efficiency and circulation number. The number and sequence of primers were found in supplemental data of Table I.

Detection of Trehalose 6,6-dibehenate (TDB) Induced Cytokine Production in Macrophages by ELISA

The cell supernatant was taken, and ELISA was used to detect the secretion of IL-1β and IL-6 in macrophages. The operation was carried out strictly according to the instructions with each sample two repeat wells, and the difference between the two was no more than 20%.

Nitric Oxide (NO) Experiment

The macrophages were stimulated with 100 ng/mL LPS and 20 μg/mL TDB respectively. The cell supernatant was collected after 96 hours, and the production of NO was detected by Griess reaction.

Statistical Analysis

The measured data were expressed as mean ± standard deviation ($\bar{x}$ + SD), and statistical product and service solutions (SPSS18.0, SPSS Inc., Chicago, IL, USA) software was used for statistical analysis. Kolmogorov-Smirnov method was used to test whether the data in each group was in accordance with a normal distribution. Single factor analysis of variance was used when the data fitted normal distribution, while the non-parametric test was used if the data did not fit. LSD method (homogeneity of variance) and Tamhane T2 (variance inhomogeneous) were used for multiple comparisons of single factor analysis of variance, and $p < 0.05$ was considered statistically different.

Results

Identification of Mice Genotypes

The PKC δ gene was amplified by PCR, then 1% agarose gel electrophoresis was performed to observe the target DNA bands. Wild-type DNA strip was 2400bp in size, and the PKC δ knockout DNA strip is 1300 bp. The experimental results are in accordance with the predicted size (Figure 1A).

Table I. Numbers and sequences of primers used in PCR experiment.

Primer name	Primer's sequences (5'-3')
m-GAPDH SF	AGG TCG GTG TGAACG GAT TTG
m-GAPDH SR	TGT AGA CCA TGT AGT TGA GGT CA
m-IL-1β SF	GCAACT GTT CCT GAA CTC AAC T
m-IL-1β SR	ATC TTT TGG GGT CCG TCAACT
m-IL-6 SF	TAG TCC TTC CTA CCC CAA TTT CC
m-IL-6 SR	TTG GTC CTT AGC CAC TCC TTC
m-iNOS SF	CGAAAC GCT TCA CTT CCAA
m-iNOS SR	TGA GCCTAT ATT GCT GTG GCT

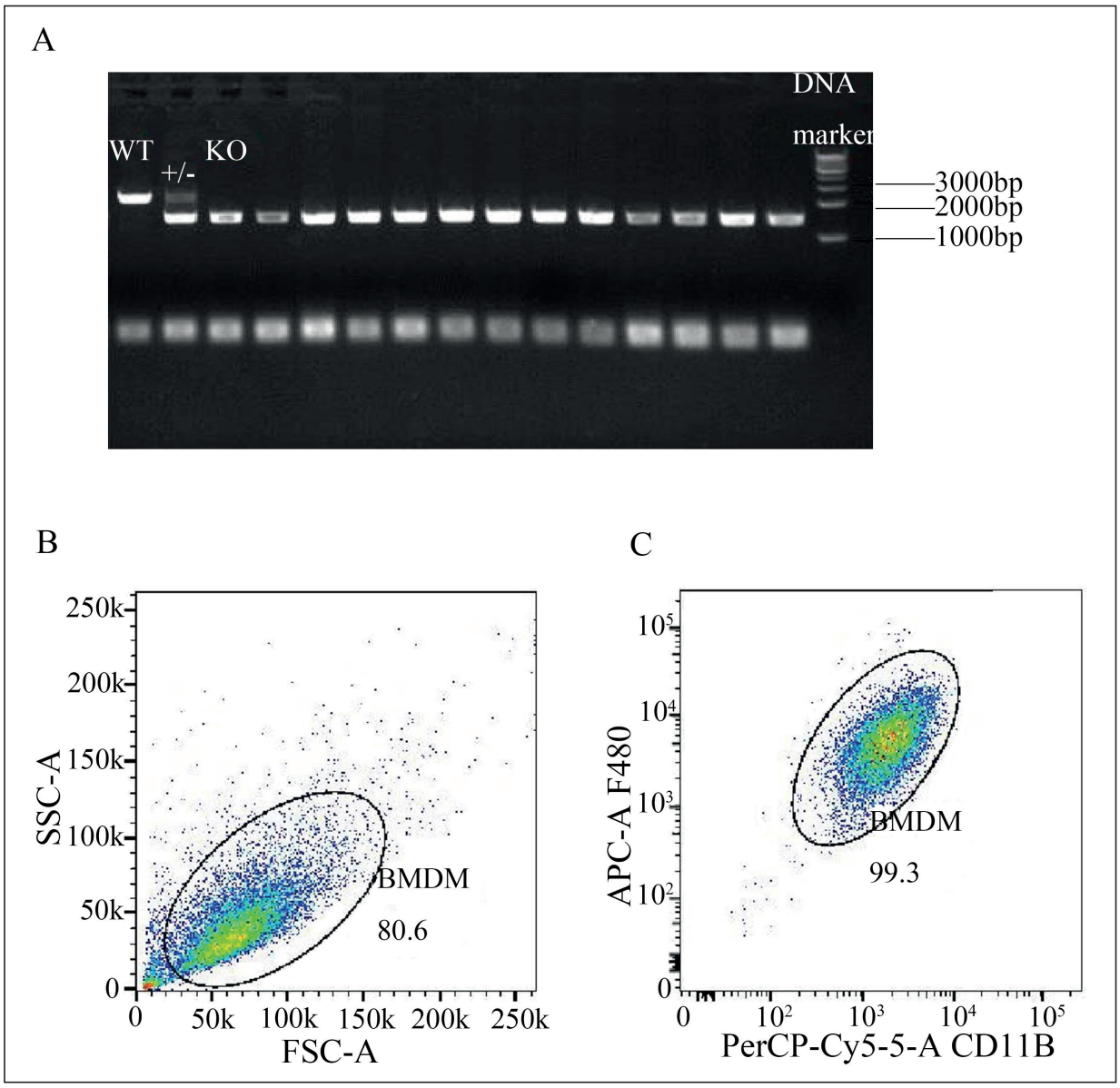

Figure 1. Identification of genotypes of experimental mice and identification of macrophage by flow cytometry. ***A,*** PCR identification mouse genotype results. DNA marker: 1 KB; WT: wild type mice; +/-: heterozygous mice; KO: PKC δ knockout mice. ***B,*** Identification of living cell concentration by flow cytometry. ***C,*** Identification of the purity of macrophages by flow cytometry.

Identification of Cell Purity of L929 Induced Bone Marrow-Derived Primary Macrophages

After bone marrow cells were induced by L929 supernatant for seven days, the mature macrophages were stained with specific flow antibody anti-CD11b-Per CP-cy5-5 and anti-F480-APC, and the results were analyzed by flow cytometry and Flow Jo software (Figure 1B-C). The experimental results showed that the number of living cells accounted for 80.6%, and the macrophages accounted for 99.3% of the total number of living cells. Therefore, we suggested that using L929 supernatant to induce bone marrow cells to differentiate into macrophages could achieve a high purity of macrophages, which was a desirable method.

PKC δ Regulates the Secretion of TDB Induced Macrophage Cytokines

Macrophages were stimulated with 100 ng/mL LPS and 20 μg/mL TDB respectively. After

24 hours of stimulation, the cell supernatant was collected and the production of IL-1β and IL-6 was detected by ELISA. The experimental results showed that under the stimulation of LPS, wild-type and PKC δ-/- macrophage presented the same cytokine secretion level (Figure 2A&B). However, after TDB stimulation, the IL-1β and IL-6 secretion level of PKC δ-/- macrophages declined significantly compared with wild-type macrophages (Figure 2C-D).

PKC δ Regulates the Transcription of TDB Induced Macrophage Cytokines

LPS stimulated cells were used as positive control, while the cells without any stimulation were set as negative control. Macrophages were stimulated with 100 ng/mL LPS and 10 μg/mL TDB respectively. After 6 hours, the cell RNA was extracted and the transcription levels of IL-1β and IL-6 were detected by real-time PCR. The results showed that with the stimulation of LPS, wild-type and PKC δ-/- macrophages presented the same RNA transcriptional level of IL-1β and IL-6 (Figure 3A-3B). After TDB stimulation, the mRNA levels of IL-1β and IL-6 in PKC δ-/- macrophages reduced significantly compared with wild-type macrophages (Figure 3C-3D).

PKC δ Regulates the Release of NO from Macrophages Induced by TDB

Similarly, LPS stimulated cells were still used as a positive control and the cells without any stimulation were set as negative control. Macrophages were stimulated with 100 ng/mL LPS and 20 μg/mL TDB respectively, and the cell supernatant was collected after 96 hours. Then Griess reaction was performed to detect the production of NO. The experimental results indicated that with the stimulation of LPS, the NO production of wild-type and PKC δ-/- macrophages was consistent (Figure 4A). However, with TDB stimulation, the NO production of PKC δ-/- macrophages

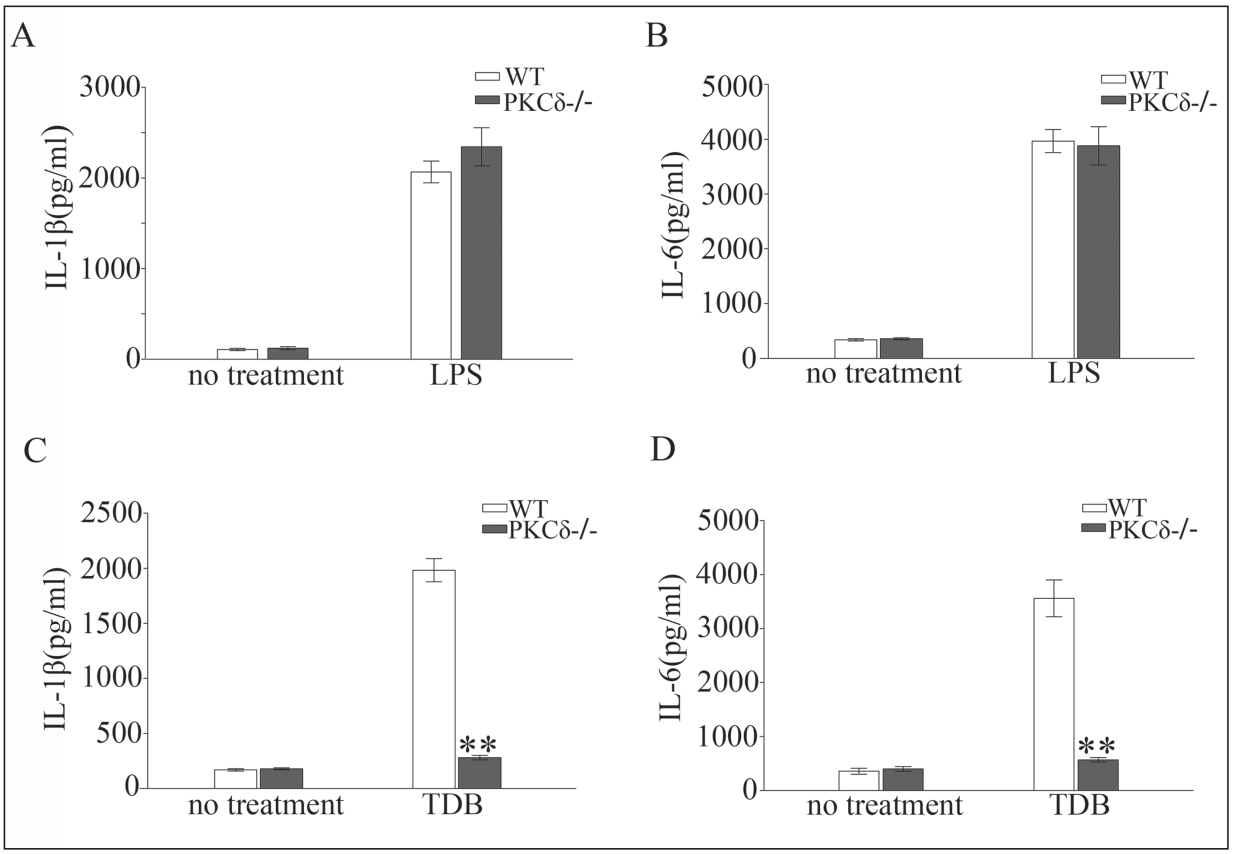

Figure 2. Production of macrophage inflammatory factors in different genotypes of mice. ***A,*** Secretion of IL-1β in macrophages after LPS stimulation. ***B,*** Production of IL-6 in macrophages after LPS stimulation. ***C,*** Secretion of IL-1 β in macrophages after TDB stimulation. ***D,*** Production of IL-6 in macrophages after TDB stimulation (Note: **compared with WT mice $p < 0.01$).

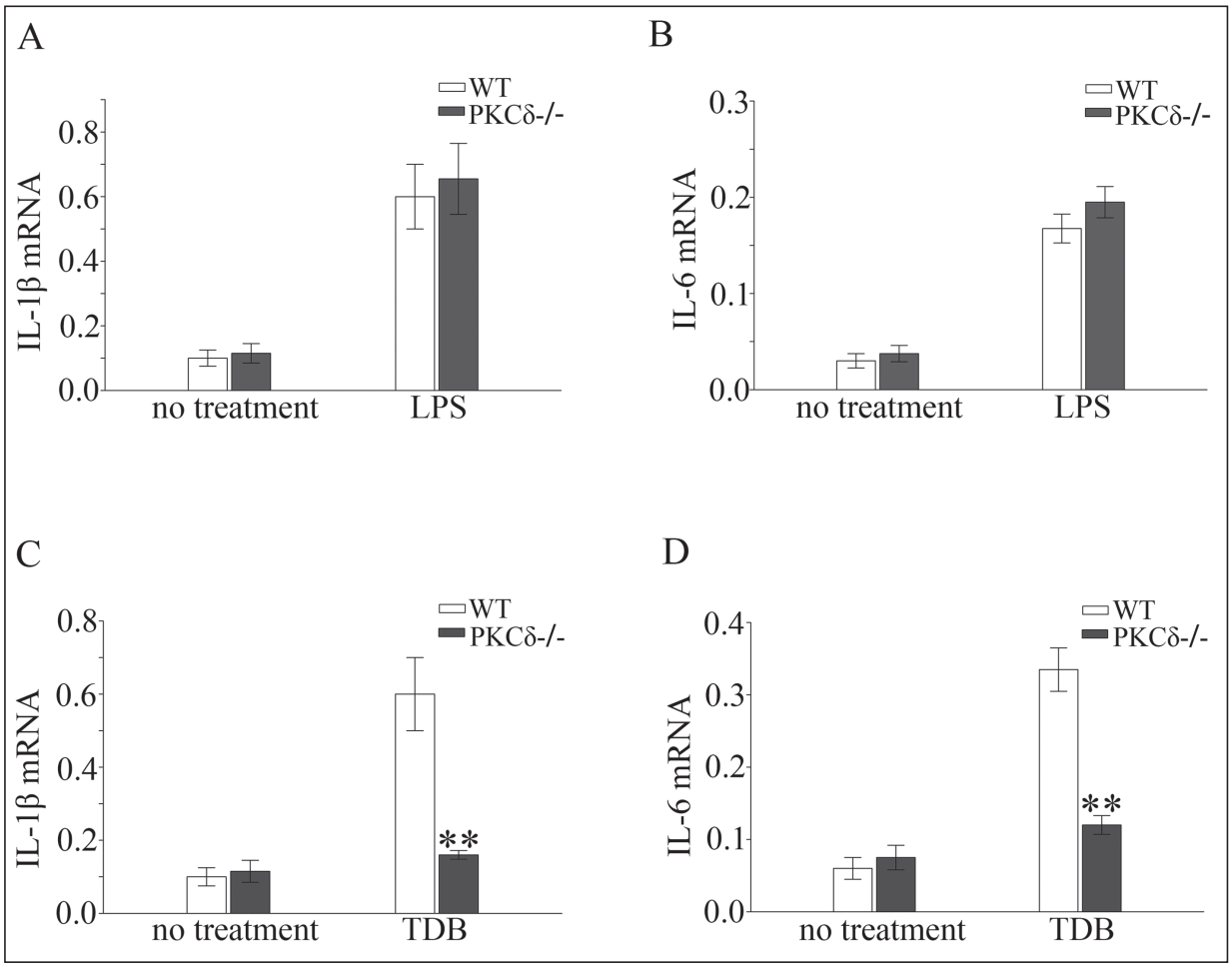

Figure 3. mRNA transcriptional level of mouse macrophages of different genotypes. ***A,*** The transcriptional level of IL-1 β mRNA in macrophages after LPS stimulation. ***B,*** The transcriptional level of IL-6 mRNA in macrophages after LPS stimulation. ***C,*** The transcriptional level of IL-1 β mRNA in macrophages after TDB stimulation. ***D,*** The transcriptional level of IL-6 mRNA in macrophages after TDB stimulation. (Note: **compared with WT mice $p < 0.01$).

decreased significantly compared with wild-type macrophages (Figure 4B). Therefore, we considered that PKC δ could regulate the TDB induced NO release of macrophages.

PKC δ Regulates TDB Induced iNOS Transcription in Macrophages

Inducible NO (iNOS) is NO synthetase in macrophages, but it does not exist when macrophages are in resting state. After macrophage was activated, iNOS gene transcription level was upregulated and a large number of NO was produced to play a role of immune killing. The macrophages were stimulated with 100 ng/m LPS and 10 μg/mL TDB respectively. RNA was extracted after 6 hours, and iNOS transcriptional level was detected by real-time PCR. It was found that with the stimulation of LPS, iNOS transcriptional level of PKC δ-/- macrophages was lower than that of wild-type macrophages, but the difference was not statistically significant (Figure 4C). Nevertheless, with TDB stimulation, the level of iNOS in wild-type macrophages increased significantly compared with PKC δ-/- macrophages (Figure 4D). Therefore, PKC δ was considered to be able to regulate the transcription level of TDB induced iNOS in the macrophage.

Discussion

Tuberculosis is a very serious infectious disease caused by *Mycobacterium tuberculosis*. After infection, the pathogen can induce the acti-

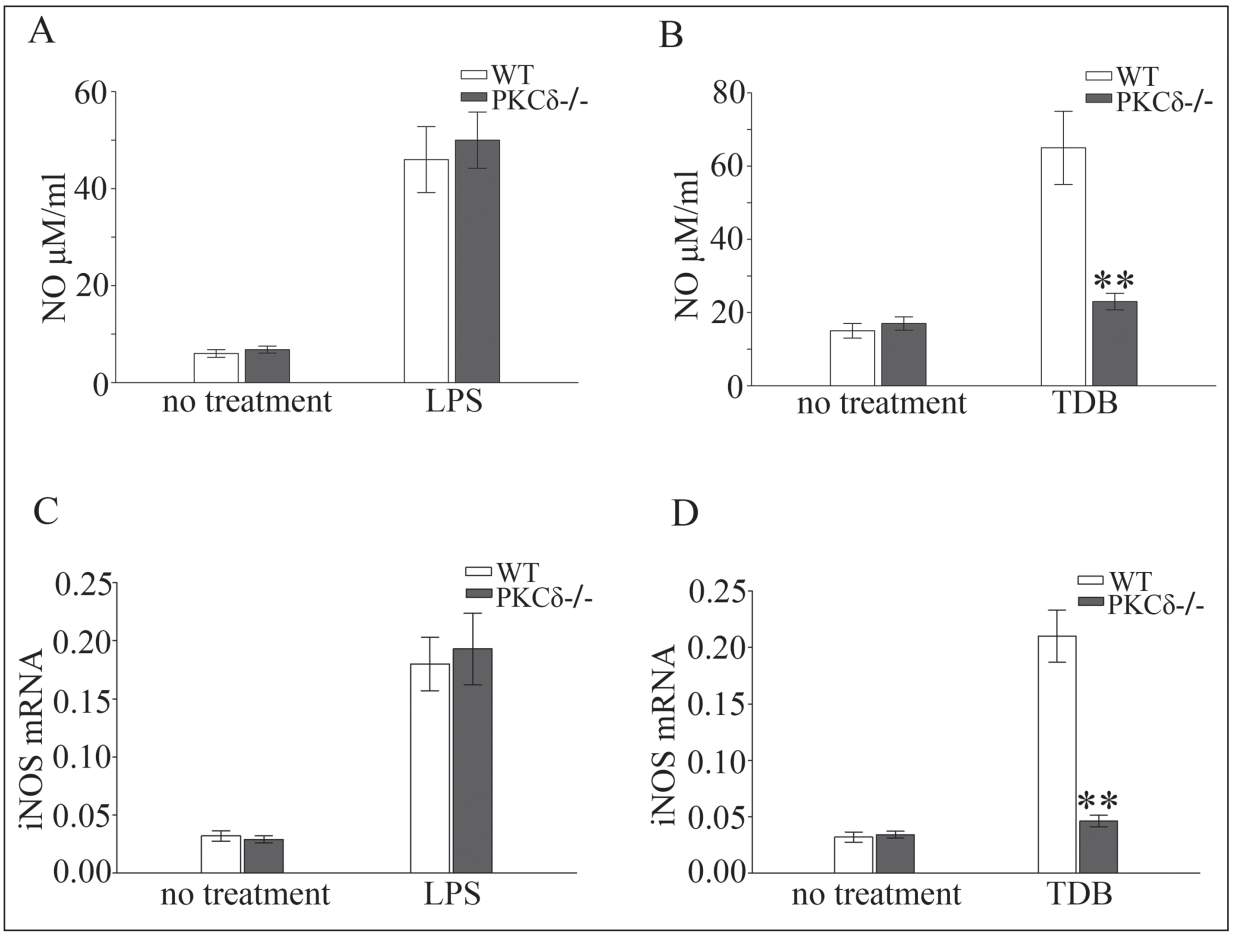

Figure 4. Production of NO and iNOS mRNA transcriptional level in macrophages of different genotypes of mice. ***A,*** The production of NO in macrophages after LPS stimulation. ***B,*** The production of NO in macrophages after TDB stimulation. ***C,*** Transcription of iNOS m RNA in macrophages after LPS stimulation. ***D,*** The transcriptional level of iNOS m RNA in macrophages after TDB stimulation. (Note: *compared with WT mice $p < 0.01$).

vation of the immune system to identify and kill the pathogenic bacteria. The macrophage is an important member of the innate immune system, which plays an important role in the infection of *Mycobacterium tuberculosis*.

Mycobacterium tuberculosis can be recognized by the innate immune system after infection, and Macrophage, as a member of the body's innate immune cells, plays an important role in the infection of *Mycobacterium tuberculosis*[10,11]. When the body is infected by *Mycobacterium tuberculosis*, macrophages first identify and engulf the pathogens, and then the pathogenic bacteria that are not removed can lurk and parasitize in the cell of macrophages. Macrophages are the first barrier against the infection of *Mycobacterium tuberculosis*. Meanwhile, they are also the main places causing the latent infection of *Mycobacterium tuberculosis* in the body. The interaction between macrophages and *Mycobacterium tuberculosis* has a great impact on the occurrence and development of tuberculosis. Therefore, exploring the interaction between them is of great significance for the research and treatment of tuberculosis. The occurrence and effect of innate immunity also involve complex identification mechanism. Pattern-recognition receptor (PRR) is a class of recognition molecules expressed on the surface of innate immune cells, which are non-clonal and pathogen-identifiable. It can identify the important and conservative components of pathogenic micro-organisms, namely Pathogen-Associated Molecular Pattern (PAMP), thereby activating the downstream signal transduction pathway of the pattern recognition receptor and triggering the activation of the innate immune system[12-17]. TDM

is a kind of glycolipid component that exists on the surface of *Mycobacterium tuberculosis* cell wall, and is the main pathogenic substance of *Mycobacterium tuberculosis*. It can induce strong inflammatory response and make immune effector molecules and immune cells gathered in the inflammatory section to participate in the formation of pneumonia granuloma[18].

Multiple pattern recognition receptors of macrophages play an important role in the identification of *Mycobacterium tuberculosis* infection, among which C type lectin receptors (CLRs) are the most crucial. Mincle and MCL are model recognition receptors that exist on the surface of macrophages and both of them belong to the CLRs family[19-24]. Ishikawa et al[19] found that Mincle can specifically identify TDM on the cell wall of *Mycobacterium tuberculosis* through the carbohydrate recognition domain (CRD) of its extracellular region, there by activating macrophages. Activated macrophages produce a large number of inflammatory cytokines and NO through a series of intracellular signal transduction. At the same time, they can also induce a large number of immune cells and inflammatory factors to accumulate in inflammatory sites, forming typical granuloma in the lungs[25]. Recently, Miyake et al[21] and Zhao, et al[22] found that MCL plays the same role with Mincle in the above pathological process. At the same time, they also found that the expression of Mincle on macrophages was very low at resting state. When TDM was identified by MCL, it could induce the activation of NF-κB through the formation of CARD9/Bcl10/MAlT1 complex to up-regulate the expression of Mincle on the cell membrane. In 2010, Marakalala et al[26] prepared mice models with Syk and its downstream gene Card9, Bcl10 and Malt1 knockout respectively. Bone marrow-derived macrophages of the above knockout mice were stimulated by the purified TDB *in vitro*. They found that compared with the wild-type mice, both the production of various cytokines and NO in the mutant macrophages decreased significantly. At the same time, they also found that the phosphorylation of Syk was dependent on its upstream protein FcR-γ[27]. Therefore, they came to the conclusion that after TDM was identified by macrophages, Syk was activated to induce the formation of Card9-Bcl10-Malt1 complex and activate NF-κB signaling pathway, leading to a significant increased expression of inflammatory cytokines that trigger innate immune responses or activate T cells to induce adaptive immune responses[28].

In the process of anti-fungal infection, the innate immune cells also depend on the C-type lectin receptor on its surface to identify the pathogen[29,30]. Dectin-1 is one of the most important pattern recognition receptors for innate immune cells to identify fungal. It can identify specific fungal surface β-glucan and then the activated innate immune cells phosphorylate the ITAM of immune receptor in the cytoplasm. Through a series of signal transduction pathways, NO and various cytokines were produced for activating adaptive immune response to play the role of anti-fungal infection[31]. Zhu et al[32] also found that MCl and Dectin2 could form heterogeneous dimers in the process of anti-fungal infection and identify the α-mannan on the surface of *Candida albicans* to activate the innate immune cells and initiate the innate immune response. It was found in the latest study that dendritic cells can activate PKC δ after identifying fungi and induce the formation of the Card9-Bcl10 complex to phosphorylate the downstream TAK1. As a result, the NF-κB signaling pathway was activated, and the release of pro-inflammatory cytokine was ultimately promoted[33].

TDM, as a kind of glycolipid component, exists on the surface of *Mycobacterium tuberculosis* cell wall. Additionally, TDM is the main pathogenic substance of *Mycobacterium tuberculosis*, which can induce a strong inflammatory response and gather immune effector molecules as well as immune cells in the inflammatory part so as to participate in the formation of pneumonia granuloma[34]. Trehalose-6,6-dibehenate (TDB) is an *in vitro* synthetic product of TDM. Researchers found that when TDB was used to replace TDM to stimulate mice, it could produce the same immune response[35].

Our study showed that, in macrophages of PKC δ knockout mice, IL-1β and IL-6 mRNA transcription and protein expression levels were significantly decreased after TDB stimulation, indicating that PKC δ was involved in the TDB-induced production of macrophage cytokines. NO produced by macrophages can kill pathogenic bacteria and has an immunomodulatory effect. It was found that, after TDB stimulation, the production of iNOS and NO in macrophages of PKC δ knockout mice also showed a significant decline, indicating that the killing function of macrophages in PKC δ knockout mice declined. Therefore, we believe that the PKC δ gene is involved in TDM-induced pulmonary inflammation. Through the experimental results, we

believe that the PKC δ gene exists in the signal transduction pathway of TDM-induced anti-tuberculosis ability of macrophages macrophage anti-MTB macrophage resistant *Mycobacterium tuberculosis* infection.

Conclusions

We showed that PKC δ was involved in the process of macrophages against the infection of *Mycobacterium tuberculosis*. In addition, PKC δ can affect macrophage's immune function by regulating the production of various cytokines and effector molecules, thereby regulating the innate immune response of the body to enhance the anti-tuberculosis ability of macrophages.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Yu WY, Lou C, Liu FJ, He DW. Clinical efficacy of one stage posterior debridement joint graft fixation for lumbar vertebral fractures in spinal tuberculosis patients with compression. Eur Rev Med Pharmacol Sci 2016; 20: 3161-3167.
2) Cole J, Aberdein J, Jubrail J, Dockrell DH. The role of macrophages in the innate immune response to Streptococcus pneumoniae and Staphylococcus aureus: Mechanisms and contrasts. Adv Microb Physiol 2014; 65: 125-202.
3) Rajaram MV, Ni B, Dodd CE, Schlesinger LS. Macrophage immunoregulatory pathways in tuberculosis. Semin Immunol 2014; 26: 471-485.
4) Xu G, Wang J, Gao GF, Liu CH. Insights into battles between Mycobacterium tuberculosis and macrophages. Protein Cell 2014; 5: 728-736.
5) An Q, Jin MW, An XJ, Xu SM, Wang L. Macrophage activation syndrome as a complication of juvenile rheumatoid arthritis. Eur Rev Med Pharmacol Sci 2017; 21: 4322-4326.
6) Duquesnes N, Lezoualc'h F, Crozatier B. PKC-delta and PKC-epsilon: foes of the same family or strangers? J Mol Cell Cardiol 2011; 51: 665-673.
7) Mellor H, Parker PJ. The extended protein kinase C superfamily. Biochem J 1998; 332 (Pt 2): 281-292.
8) Li L, Okusa MD. Macrophages, dendritic cells, and kidney ischemia-reperfusion injury. Semin Nephrol 2010; 30: 268-277.
9) Bey EA, Xu B, Bhattacharjee A, Oldfield CM, Zhao X, Li Q, Subbulakshmi V, Feldman GM, Wientjes FB, Cathcart MK. Protein kinase C delta is required for p47phox phosphorylation and translocation in activated human monocytes. J Immunol 2004; 173: 5730-5738.
10) Sia JK, Georgieva M, Rengarajan J. Innate immune defenses in human tuberculosis: an overview of the interactions between mycobacterium tuberculosis and innate immune cells. J Immunol Res 2015; 2015: 747543.
11) Lerner TR, Borel S, Gutierrez MG. The innate immune response in human tuberculosis. Cell Microbiol 2015; 17: 1277-1285.
12) Jeannin P, Magistrelli G, Goetsch L, Haeuw JF, Thieblemont N, Bonnefoy JY, Delneste Y. Outer membrane protein a (OmpA): a new pathogen-associated molecular pattern that interacts with antigen presenting cells-impact on vaccine strategies. Vaccine 2002; 20 Suppl 4: A23-A27.
13) Schuberth HJ, Dahnke J, Leibold W, Seyfert HM, Zerbe H. [Bacterial pathogen associated molecular pattern and superantigens indirectly induce the accelerated death of bovine neutrophilic granulocytes]. Berl Munch Tierarztl Wochenschr 2004; 117: 464-471.
14) Vuong C, Durr M, Carmody AB, Peschel A, Klebanoff SJ, Otto M. Regulated expression of pathogen-associated molecular pattern molecules in Staphylococcus epidermidis: quorum-sensing determines pro-inflammatory capacity and production of phenol-soluble modulins. Cell Microbiol 2004; 6: 753-759.
15) Mishina TE, Zeier J. Pathogen-associated molecular pattern recognition rather than development of tissue necrosis contributes to bacterial induction of systemic acquired resistance in Arabidopsis. Plant J 2007; 50: 500-513.
16) Chintakuntlawar AV, Zhou X, Rajaiya J, Chodosh J. Viral capsid is a pathogen-associated molecular pattern in adenovirus keratitis. PLoS Pathog 2010; 6: e1000841.
17) Stone AE, Giugliano S, Schnell G, Cheng L, Leahy KF, Golden-Mason L, Gale MJ, Rosen HR. Hepatitis C virus pathogen associated molecular pattern (PAMP) triggers production of lambda-interferons by human plasmacytoid dendritic cells. PLoS Pathog 2013; 9: e1003316.
18) Hunter RL, Olsen MR, Jagannath C, Actor JK. Multiple roles of cord factor in the pathogenesis of primary, secondary, and cavitary tuberculosis, including a revised description of the pathology of secondary disease. Ann Clin Lab Sci 2006; 36: 371-386.
19) Ishikawa E, Ishikawa T, Morita YS, Toyonaga K, Yamada H, Takeuchi O, Kinoshita T, Akira S, Yoshikai Y, Yamasaki S. Direct recognition of the mycobacterial glycolipid, trehalose dimycolate, by C-type lectin Mincle. J Exp Med 2009; 206: 2879-2888.
20) Schoenen H, Bodendorfer B, Hitchens K, Manzanero S, Werninghaus K, Nimmerjahn F, Agger EM, Stenger S, Andersen P, Ruland J, Brown GD, Wells C, Lang

R. Cutting edge: mincle is essential for recognition and adjuvanticity of the mycobacterial cord factor and its synthetic analog trehalose-dibehenate. J Immunol 2010; 184: 2756-2760.

21) Miyake Y, Toyonaga K, Mori D, Kakuta S, Hoshino Y, Oyamada A, Yamada H, Ono K, Suyama M, Iwakura Y, Yoshikai Y, Yamasaki S. C-type lectin MCL is an FcRgamma-coupled receptor that mediates the adjuvanticity of mycobacterial cord factor. Immunity 2013; 38: 1050-1062.

22) Zhao XQ, Zhu LL, Chang Q, Jiang C, You Y, Luo T, Jia XM, Lin X. C-type lectin receptor dectin-3 mediates trehalose 6,6'-dimycolate (TDM)-induced Mincle expression through CARD9/Bcl10/MALT1-dependent nuclear factor (NF)-kappaB activation. J Biol Chem 2014; 289: 30052-30062.

23) Yonekawa A, Saijo S, Hoshino Y, Miyake Y, Ishikawa E, Suzukawa M, Inoue H, Tanaka M, Yoneyama M, Oh-Hora M, Akashi K, Yamasaki S. Dectin-2 is a direct receptor for mannose-capped lipoarabinomannan of mycobacteria. Immunity 2014; 41: 402-413.

24) Lobato-Pascual A, Saether PC, Fossum S, Dissen E, Daws MR. Mincle, the receptor for mycobacterial cord factor, forms a functional receptor complex with MCL and FcepsilonRI-gamma. Eur J Immunol 2013; 43: 3167-3174.

25) Bekierkunst A, Levij IS, Yarkoni E, Vilkas E, Adam A, Lederer E. Granuloma formation induced in mice by chemically defined mycobacterial fractions. J Bacteriol 1969; 100: 95-102.

26) Marakalala MJ, Graham LM, Brown GD. The role of Syk/CARD9-coupled C-type lectin receptors in immunity to Mycobacterium tuberculosis infections. Clin Dev Immunol 2010; 2010: 567571.

27) Gross O, Gewies A, Finger K, Schafer M, Sparwasser T, Peschel C, Forster I, Ruland J. Card9 controls a non-TLR signalling pathway for innate anti-fungal immunity. Nature 2006; 442: 651-656.

28) Shenderov K, Barber DL, Mayer-Barber KD, Gurcha SS, Jankovic D, Feng CG, Oland S, Hieny S, Caspar P, Yamasaki S, Lin X, Ting JP, Trinchieri G, Besra GS, Cerundolo V, Sher A. Cord factor and peptidoglycan recapitulate the Th17-promoting adjuvant activity of mycobacteria through mincle/CARD9 signaling and the inflammasome. J Immunol 2013; 190: 5722-5730.

29) Ferwerda B, Ferwerda G, Plantinga TS, Willment JA, van Spriel AB, Venselaar H, Elbers CC, Johnson MD, Cambi A, Huysamen C, Jacobs L, Jansen T, Verheijen K, Masthoff L, Morre SA, Vriend G, Williams DL, Perfect JR, Joosten LA, Wijmenga C, van der Meer JW, Adema GJ, Kullberg BJ, Brown GD, Netea MG. Human dectin-1 deficiency and mucocutaneous fungal infections. N Engl J Med 2009; 361: 1760-1767.

30) Taylor PR, Tsoni SV, Willment JA, Dennehy KM, Rosas M, Findon H, Haynes K, Steele C, Botto M, Gordon S, Brown GD. Dectin-1 is required for beta-glucan recognition and control of fungal infection. Nat Immunol 2007; 8: 31-38.

31) Wevers BA, Kaptein TM, Zijlstra-Willems EM, Theelen B, Boekhout T, Geijtenbeek TB, Gringhuis SI. Fungal engagement of the C-type lectin mincle suppresses dectin-1-induced antifungal immunity. Cell Host Microbe 2014; 15: 494-505.

32) Zhu LL, Zhao XQ, Jiang C, You Y, Chen XP, Jiang YY, Jia XM, Lin X. C-type lectin receptors Dectin-3 and Dectin-2 form a heterodimeric pattern-recognition receptor for host defense against fungal infection. Immunity 2013; 39: 324-334.

33) Strasser D, Neumann K, Bergmann H, Marakalala MJ, Guler R, Rojowska A, Hopfner KP, Brombacher F, Urlaub H, Baier G, Brown GD, Leitges M, Ruland J. Syk kinase-coupled C-type lectin receptors engage protein kinase C-sigma to elicit Card9 adaptor-mediated innate immunity. Immunity 2012; 36: 32-42.

34) Abbott AN, Guidry TV, Welsh KJ, Thomas AM, Kling MA, Hunter RL, Actor JK. 11Beta-hydroxysteroid dehydrogenases are regulated during the pulmonary granulomatous response to the mycobacterial glycolipid trehalose-6,6'-dimycolate. Neuroimmunomodulat 2009; 16: 147-154.

35) Desel C, Werninghaus K, Ritter M, Jozefowski K, Wenzel J, Russkamp N, Schleicher U, Christensen D, Wirtz S, Kirschning C, Agger EM, Prazeres DCC, Lang R. The Mincle-activating adjuvant TDB induces MyD88-dependent Th1 and Th17 responses through IL-1R signaling. Plos One 2013; 8: e53531.