The Mechanism of *Talaromyces Marneffei* Influencing Macrophage RAW264.7 Polarization and Sterilization Ability via Arginine Metabolism Pathway

Linxia Shen

Huashan Hospital Fudan University

Di Yang

Guangxi Medical University First Affiliated Hospital

Rifeng Chen

Guangxi Medical University First Affiliated Hospital

Donghua Liu (✉ ldhgxm@163.com)

Guangxi Medical University First Affiliated Hospital <https://orcid.org/0000-0002-5296-7665>

Research article

Keywords: *Talaromyces marneffei*, macrophage RAW264.7, Arginase, iNOS, Arginine

Posted Date: September 3rd, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-48911/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

The opportunistic fungi *T. marneffe* is an intracellular pathogen that causes systemic and lethal infection through the mononuclear phagocyte system. Macrophages act as pacesetters to resist pathogen invasion and communicators to activate adaptive immunity. During infection, macrophages induce iNOS to generate NO, which kills invading pathogenic microorganisms. Arginase competes with the iNOS for a common substrate L-arginine. We tried to investigate the effect of arginine metabolism on the escape of *T. marneffe* from macrophage killing.

Results

We performed qPCR, immunoblot detection, immunofluorescent staining, arginase activity assay and NO production assay when macrophages were co-cultured with *T. marneffe*. We conducted immunoblot detection, arginase activity assay, NO production assay, phagocytic activity assay and *T. marneffe* killing assay after adding the arginase specific inhibitor nor-NOHA to the co-culture system. *T. marneffe* decreased the NO production in macrophages by increasing macrophage arginase activity. With lastingness of infection, *T. marneffe* induced macrophages towards M2 phenotype polarization, thereby reducing antimicrobial activity and promoting fungal survival. Moreover, inhibiting the *T. marneffe*-induced macrophage arginase activity with nor-NOHA restored NO synthesis and strengthened the function of fungal killing.

Conclusions

T. marneffe potentiated its own survival via regulating arginine metabolism pathway in macrophages during an infection.

Background

Penicilliosis marneffe is a fungal disease caused by *Talaromyces marneffe* (*T. marneffe*) which is regarded as a temperature-dependent dimorphic opportunity pathogenic fungus. *Penicilliosis marneffe* was rare in the past. Since the increasing number of population with HIV, and the wide use of glucocorticoid and immunosuppressor, *T. marneffe* infection has been the most common systemic fungal infection in Southeast Asia and southern China [1]. Up to now, the pathway of *T. marneffe* infection has been controversial. Hamilton et al thought *T. marneffe* infection was presumed to originate in the lung after inhalation of the airborne conidia into alveoli, which were engulfed by macrophages after combining with laminin [2]. Via the phagocyte system, *T. marneffe* causes the fatal disseminated infection in immunocompromised/healthy individuals. However, the mechanism that *T. marneffe* successfully replicates and escapes from the immune system has not been completely clear. The

treatment of *T. marneffe*i infection is still in the exploratory stage. We fail to find a better way to fight the infection except anti-fungal drugs.

Macrophages participate in the innate and adaptive immunity, and play an essential role in internal environmental homeostasis. Under different pathophysiological conditions, they present distinct functional polarization phenotypes [3]. Classically activated M1 macrophages, induced by IFN- γ or LPS, express the pro-inflammatory mediators (TNF- α , IL-1, IL-6, NO and ROS), which are the key effectors against the microbicidal and tumoricidal activity. In contrast, the alternatively activated M2 macrophages, induced by IL-4 or IL-13, express the anti-inflammatory mediators which mediate the Th2 response and tissue repair.

The focus of macrophage polarization is the arginine metabolism pathway [4]. NOS hydrolyzes the arginine as NO and L-ornithine. As a key component of innate immune response in macrophages, NO possesses a strong sterilization ability against pathogens, especially the intracellular pathogens like mycobacterium tuberculosis, leishmania and salmonella. Arginase is a competitive inhibitor of NOS, which hydrolyzes the arginine into urea and L-ornithine, limiting the excessive production of NO. Many pathogens affect the macrophage polarization by interfering with the arginine metabolism pathway as their survival strategy. They increase the arginase activity in themselves or affect the host arginase activity, thereby consuming the substrate arginine, competitively inhibiting the production of NO, inducing macrophages towards M2 phenotype, and mediating the persistence of infection. When the specific Arg inhibitor N^w-hydroxy-nor-arginine (nor-NOHA) was used, the synthesis of NO in macrophages was restored and the antibiotic activity was enhanced [5–7]. Therefore, the arginine metabolism pathway is a key regulator of innate immune response and targeting it may better control the infection.

Although the mechanism in which *T. marneffe*i evades the host immunity remains unknown, it might be a new method to treat *T. marneffe*i infection by intervening the arginine metabolism pathway of macrophages.

Results

*T. marneffe*i utilized the arginine metabolism pathway of macrophages

We hypothesized that *T. marneffe*i promoted its survival in macrophages by utilizing the arginine metabolism pathway. Therefore, we analyzed the Arg1, iNOS mRNA and protein expressions in M ϕ and LPS-induced macrophages co-cultured with *T. marneffe*i (MOI = 10) for 12 h, 24 h and 72 h. *T. marneffe*i significantly increased the iNOS mRNA expression in the co-culture system at 24 h, 48 h and 72 h. With the extension of co-culture time, the iNOS mRNA level increased initially and then decreased (Fig. 1A, C). However, the Arg1 mRNA expression in the co-culture system increased only at 72 h (Fig. 1B, D). Interestingly, *T. marneffe*i significantly increased the iNOS protein expression similarly at 24 h, 48 h and 72 h (Fig. 2A-C). But *T. marneffe*i decreased the Arg1 protein level in M ϕ + TM group at 72 h, and in M ϕ + LPS + TM group at 48 h and 72 h (Fig. 2D-F).

Simultaneously, we examined the arginase activity of macrophages and the concentration of NO in the supernatant. *T. marneffei* markedly increased the arginase activity at 24 h, 48 h and 72 h. The longer the co-culture period was, the higher the arginase activity was in the Mφ + LPS + TM group (Fig. 3A, C). The NO synthesis decreased at 24 h, 48 h and 72 h, but there was no difference among the three time points (Fig. 3B, D).

***T. marneffei* affected the macrophage polarization**

To explore the effect of *T. marneffei* on macrophage polarization, we analyzed the mRNA expressions of TNF-α, IL-1β, CD301 and IL-10 in Mφ or LPS-activated macrophages co-cultured with *T. marneffei* (MOI = 10) for 12 h, 24 h and 72 h. *T. marneffei* significantly increased the mRNA expressions of TNF-α and IL-1β in co-culture system at 24 h, 48 h and 72 h. With the extension of co-culture time, the TNF-α and IL-1β mRNA expressions in the Mφ + TM group gradually increased (Fig. 4A, B). Moreover, the TNF-α mRNA expression in the Mφ + LPS + TM group showed a trend of fall-rise (Fig. 4E), while the IL-1β mRNA expression increased and then decreased (Fig. 4F). In addition, *T. marneffei* significantly increased the IL-10 mRNA level in the Mφ + TM group at 24 h, 48 h and 72 h, while there was no difference among 3 time points (Fig. 4C). *T. marneffei* also elevated the IL-10 mRNA level in the Mφ + LPS + TM group at 24 h and 48 h (Fig. 4G). The CD301 mRNA expression in co-culture system increased at 48 and 72 h. With the extension of co-culture time, the CD301 mRNA level in the Mφ + LPS + TM group gradually increased (Fig. 4D, H).

Simultaneously, we also analyzed the CD86, IL-4R and CD206 protein expressions. CD86 is a marker of M1 polarization. IL-4R and CD206 are markers of M2 polarization. The results showed that *T. marneffei* reduced the CD86 protein expression at 72 h and increased the IL-4R protein expression at 72 h (Fig. 5A-D). Moreover, the fluorescence intensity of CD206 in macrophages co-cultured with *T. marneffei* (MOI = 10) for 72 h was greater than the Mφ/Mφ + LPS group (Fig. 5E).

Effect of nor-NOHA on the arginine metabolism pathway in co-culture system

To explore the effect of nor-NOHA on macrophages against *T. marneffei* infection, we studied the effect of nor-NOHA on the arginine metabolism pathway in co-culture system. The Arg1 and iNOS protein expressions of macrophages, which were pretreated with 20 μM nor-NOHA and then co-cultured with *T. marneffei* for 24 h (MOI = 10), were detected by immunoblotting. The results indicated that nor-NOHA significantly decreased the Arg1 protein expression and increased the iNOS protein expression (Fig. 6A, B). Furthermore, we analyzed the arginase activity of macrophages and NO production in the co-culture supernatant. 20 μM nor-NOHA markedly inhibited the arginase activity in the co-culture system (Fig. 6C), and rescued the NO production up to nearly 50% (Fig. 6D).

Nor-NOHA promoted the antimicrobial function of macrophages

Next, we determined the impact of nor-NOHA on the antimicrobial function of macrophages. Inhibition of arginase activity with 20 μM nor-NOHA significantly increased the intake of *T. marneffei* which was killed

by LPS-activated macrophages (Fig.7A-C). The colony count was obviously higher in the co-cultured system than that in the *T. marneffei* group (Fig.7B, D). Besides, the colony count was lower in the co-cultured system with 20μM nor-NOHA than that in the *T. marneffei* group (Fig.7B, E).

Discussion

The objective of our study was to dissect the arginine metabolism pathway on regulating the immune function of macrophages against *T. marneffei* infection. According to the results, we found that *T. marneffei* limited the NO production in macrophages by enhancing the activity of arginase, thereby attenuating the antimicrobial function of macrophages. The mRNA expressions of TNF-α, IL-1β, IL-10 and CD301 in the co-culture system were higher than the control group. With the extension of co-culture time, the protein expression of CD86 in the co-culture system was lower than the control group while the CD206 and IL-4R protein expressions were higher. It indicated that *T. marneffei* caused the acute inflammation and concurrently mediated the anti-inflammatory response after being engulfed by macrophages. It was a dynamic process. As the immune response progressed, the inflammatory cytokines increased while the anti-inflammatory factors decreased. CD86 is a member of immunoglobulin superfamily, and its co-stimulatory signal pathway with CD80 promotes the proliferation of T lymphocytes, which play a vital role in clearing the pathogens and maintaining the balance of immune system[8]. Pathogenic microorganism inhibits the T lymphocyte function by down-regulating the expression of CD86, thus disrupting the immunoreaction, making the host unable to eliminate the pathogens effectively, and leading to the persistent infection. CD206 is known as the mannose receptor which mediates the internalization of viruses, bacteria and fungi, facilitating antigen uptake and processing in the adaptive immune response, as well as mediating direct uptake of pathogens in the innate immune response[9]. The activation of CD206 could affect the maturation of phagosomes and provide the favorable condition for the survival of pathogens in macrophages by restricting the fusion of phagosomes and lysosomes. [10, 11]. IL-4R is a specific receptor of interleukin-4. After binding to IL-4R, IL-4 exerts its biological function via the Ras pathway, PI3K pathway, IRS1/2 pathway and Jak-STAT pathway. A study showed that viruses caused the transfer of CD8⁺T cells by encoding various types of cytokines. CD8⁺T cells produced IL-4/IL-3 which suppressed the anti-viral immune response of Th1 cells [12]. According to the results, we inferred that when *T. marneffei* was engulfed by macrophages, macrophages transformed to M2 phenotype polarization by increasing the activity of arginase, so as to protect *T. marneffei* from immune killing. The severity of *T. marneffei* infection depended on its virulence and the host immune state. *T. marneffei* not only used the arginine metabolism pathway to escape the oxidative killing, but also affected the macrophage polarization balance of M1/M2. The polarization imbalance provided the favorable condition for its sustainable survival in host. This finding might provide a new theory for understanding the mechanism of *T.marneffei* resisting the host immune response.

In addition, we noticed that there was a difference between the Arg1 mRNA expression and the Arg1 protein expression when macrophages were co-cultivated with *T.marneffei*. The co-culture period of our study was 24–72 h while the co-culture period of other researches was generally less than 12 h, which

might lead to the different results from ours. Besides, we speculated that it might be the excessive increase of arginase activity, causing the arginine to react with substrate rapidly and to be consumed, thus reducing the protein expression level. The immune regulation of macrophages was complex, and there might be other mechanisms of anti-*T.marneffe*i infection, so that the increased Arg1 mRNA could not successfully convert into protein, maintaining the M1/M2 polarization balance.

In recent years, it has been found that the expression of arginase in macrophages was up-regulated in the studies of fungal infection, parasite infection, bacterial infection and other diseases [13–16]. Some scholars have proposed that the use of arginase inhibitors could reverse the pathology of diseases. As a specific inhibitor of arginase, nor-NOHA can effectively inhibit the activity of arginase without inhibiting the up-regulation of iNOS. The application of nor-NOHA has been studied in some patients with hypertension, coronary disease, heart failure and familial hypercholesterolemia, and has presented great achievements [17–20]. However, in the cases of fungal infection, there were few studies on using nor-NOHA as a means of fighting infection.

Our experimental results indicated that nor-NOHA could enhance the phagocytosis of *T.marneffe*i conidia by macrophages. Nor-NOHA inhibited the increase of arginase activity and up-regulated the expression of iNOS, so as to promote the production of abundant NO and to strengthen the sterilization ability of macrophages. Compared with the untreated *T.marneffe*i group, *T.marneffe*i pretreated with 20 μ M nor-NOHA showed the lower colony count. It demonstrated that the arginase inhibitor nor-NOHA could suppress *T.marneffe*i proliferation. Some studies reported that *Spirillum*, *Mycobacterium*, *Salmonella*, *Schistosoma*, *Trypanosoma*, *Leishmania* and *Candida* could code their arginase [21]. The arginase of *Leishmania* increased after treatment of IL-4, which promoted the growth of *Leishmania*. Moreover, the growth of *Leishmania* was also inhibited by the inhibition of arginase, and this growth inhibition was attributed to the reduction of ornithine availability [22]. The arginase encoded by *rocF* of *Helicobacter pylori* could protect it from acid stress. In a mouse model, the arginase-deficient strain of *Helicobacter pylori* showed a weakened colonization [23]. The arginase of *Helicobacter pylori* could also damage the function of T cells by reducing the expression of CD3 ζ chain [24]. *Candida albicans* yeast cells rapidly up-regulated the arginase encoded by *Car1p* after being phagocytosed by macrophages, which metabolized L-arginine to ornithine and urea, and then the urea was degraded into CO₂ and NH₃ through urea amidolyase. CO₂ activated adenylate cyclase and cAMP-dependent protein kinase A pathway (PKA), thus activating *Efg1p* and inducing *Candida albicans* to transform from yeast to hypha, so that fungi could penetrate the cell membrane and escape killing by macrophages [25]. These studies showed that some pathogenic microorganisms could increase their arginase, thus promoting their proliferation and survival in cells. *T.marneffe*i whole-genome sequencing showed that there were some genes encoding arginase, urea amidolyase and adenylate cyclase. *T.marneffe*i is an asexual reproductive fungi. The key factor of asexual development is *GasA* and the activation of *GasA* signal may involve the regulation of cAMP-PKA pathway [26]. Therefore, we speculated that *T.marneffe*i might up-regulate its arginase and urea amidolyase to produce a large amount of CO₂, which activated cAMP-PKA to regulate the *GasA*

signal, thereby stimulating the *T.marneffe* asexual development. Nor-NOHA suppressed the arginase of *T.marneffe* and blocked this pathway, thus inducing a low colony count.

It was worth noting that the single cultured *T.marneffe* (with or without nor NOHA) showed a lower colony count, compared with the co-culture group (*T.marneffe* co-cultured with macrophages). This suggested that the co-culture system promoted the growth of *T.marneffe*. In a study on Leishmania, it was found that Leishmania enhanced the host's arginase to obtain polyamines, and also used its own arginase to obtain polyamines, which were the necessary component for its growth [27]. The biosynthesis of polyamines in eukaryotes and protozoa is initiated by the hydrolysis of L-ornithine into putrescine via ornithine decarboxylase (ODC). Putrescine is converted into spermidine through the continuous transfer of amino propyl, and then into spermine. Putrescine, spermidine and spermine are the most common polyamines in bacteria and fungi. Except for two archaea, polyamines are identified in each organism [28, 29]. The universal existence proves its importance in organism. Polyamines are involved in many cellular processes, such as gene expression, cell proliferation, survival and stress response. The reverse-genetic study of microorganisms showed that the gene deletion or polyamine consumption in polyamine metabolism pathway had a negative effect on cell survival and proliferation [29]. In a study on the mutant *T.marneffe* strain of S-adenosylmethionine decarboxylase (SAMDC), which was the key enzyme of polyamine synthesis, it was found that the mutant strain had poor growth and sporulation capacity on the basic culture medium. However, the addition of spermidine in culture medium recovered the sporulation capacity and promoted the yeast development at 37°C [30]. We speculated that, on the one hand, *T.marneffe* could induce the increase of arginase activity of macrophages, inhibit the production of NO by the L-arginine pathway, thus weakening the killing capacity of macrophages, on the other hand, *T.marneffe* could regulate the increase of polyamine concentration in the environment to promote its proliferation.

T.marneffe is an intracellular fungi, mainly affecting the people with immune deficiency, such as Human Immunodeficiency Virus (HIV) positive people, IFN- γ antibody positive people, CD20 antibody positive people and kinase inhibition people [31, 32]. It has been reported that the Arg1 expression in lymph nodes and peripheral T cells of HIV patients was negatively correlated with the peripheral CD4⁺T cell count and positively correlated with the viral load. The expression of iNOS was positively related with the peripheral CD4⁺T cell count and negatively related with the viral load [33]. Perhaps the environment of high arginase in HIV patients was one of the reasons why they were susceptible to *T.marneffe*.

Conclusions

To conclude, *T.marneffe* can promote the expression of arginase in itself or the host to maintain survival through the arginine metabolism pathway. Nor-NOHA, an arginase inhibitor, can play an important role in macrophages against *T.marneffe* infection.

Methods

Cell medium and reagents

The murine macrophage cell line RAW264.7, obtained from American Type Culture Collection (ATCC, VA, USA) were maintained in complete Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, 100U/ml penicillin and 100mg/ml streptomycin at 37°C in a humidified atmosphere containing 5% CO₂. M1-polarized macrophages were induced by 500ng/ml LPS (Invitrogen, USA) for 24h.

T. marneffei strain and conidia preparation

The *T. marneffei* strain FRR2161 was cultured in the hypha phase for 2 weeks at 25°C onto PDA medium (6g potato extract, 20g dextrose, 20g agar). Collecting the colonies into 1×PBS buffer solution and filtering the hypha through sterile gauzes, then the conidia suspension was obtained.

Quantitative real-time PCR

Macrophages were collected with Trizol reagent (Invitrogen, USA). Total RNA was extracted and transformed into cDNA using Transcriptor First Strand cDNA Synthesis Kit (Roche, Switzerland). HieffTM qPCR SYBR[®] Green Master Mix (YEASEN Biotechnology, Shanghai, China) was used for qPCR analysis under Lightcycler 480 System. The program included 95°C for 5 min, 40 cycles of 95°C for 10s and 60°C for 30s. Data were normalized to GAPDH and calculated by the $2^{-\Delta\Delta CT}$ method. The primer sequences: GAPDH-f 5'-GGTCGGTGTGAACGGATTTG-3', GAPDH-r 5'-TGTAAGACCATGTAGTTGAGGTCA-3'; iNOS-f 5'-GAGGCCAGGAGGAGAGAGATCCG-3', iNOS-r 5'-TCCATGCAGACAACCTTGGTGTG-3'; TNF- α -f 5'-CCAAAGGGATGAGAAGTTCC-3', TNF- α -r 5'-CTCCACTTGGTGGTTTGCTA-3'; IL-1 β -f 5'-TGGCAACTGTTCTGAACTCAA-3', IL-1 β -r 5'-AGCAGCCCTTCATCTTTTGG-3'; Arg1-f 5'-AGCTCTGGGAATCTGCATGG-3', Arg1-r 5'-ATGTACACGATGTCTTTGGCAGATA-3'; CD301-f 5'-ACTGAGTTCCTGCCTCTGGT-3', CD301-r 5'-ATCTGGGACCAAGGAGAGTG-3'; IL-10-f 5'-CCCAGAAATCAAGGAGCATT-3', IL-10-r 5'-TCACTCTTCACCTGCTCCAC-3'.

Immunoblot analysis

Macrophages were lysed in RIPA buffer. The concentration of protein samples was measured using Pierce BCA Protein Assay Kit (Thermo, USA). The equal proteins were separated by 10% SDS-PAGE, subsequently transferred onto PVDF membrane. After blockage of non-specific antigens in 5% skimmed milk for 1h, the PVDF membrane was incubated with primary antibodies against Arg1(1:1000, ab124917, Abcam), iNOS(1:1000, ab178945, Abcam), IL-4R(1:500, sc-165974, Santa Cruz), CD86(1:1000, ab53004, Abcam) at 4°C overnight, followed by incubation with secondary antibodies for 1.5h at room temperature. The target proteins were visualized with the ECL system (Amersham Imager 600, GE) and analyzed by ImageJ Software. β -actin-HRP (1:10000) was used as a loading control.

Arginase activity assay

Arginase activity was determined using the arginase activity assay kit (Sigma-Aldrich). Samples were performed as the manufacture introduction.
$$\text{Activity(Units/L)} = \frac{[(A430)_{\text{sample}} - (A430)_{\text{blank}}] / [(A430)_{\text{standard}} - (A430)_{\text{water}}] \times [(1 \text{ mM} \cdot 50 \cdot 10^3) / (V \cdot T)]}{}$$

NO production assay

The production of NO was determined with assay kit for nitrite determination (Nanjing Jiancheng Bioengineering Institute, China). The co-cultured precipitation was centrifuged and discarded. The supernatant was collected. Different reagents were added according to the manufacture instruction. Reactions were then measured at 550nm using a microplate reader.

Immunofluorescent staining

Macrophages were seeded onto coverslips in a 12-well plate and cultivated overnight. After co-culturing with *T. marneffei* conidia for 72h, macrophages were washed in PBS. Cells were fixed in 4% paraformaldehyde for 15 minutes, permeabilized in 0.1% Triton X-100 and 1% bovine serum albumin for 15 minutes. All samples were incubated with primary antibody against CD206 (1:200, Abcam) at 4°C overnight and then incubated with secondary antibody for 1 hour at room temperature. Nuclei were counterstained in DAPI (1:500, Sigma) for 10 minutes.

Phagocytic activity assay

T. marneffei conidia suspension was prestained with Calcofluor white (Sigma, USA) and 10% KOH for 10 min. Macrophages were added on coverslips, pretreated with 20μM nor-NOHA for 2h. Following coincubation with prestained conidia suspension (MOI=10) for 2h at 37°C, samples were washed gently to remove non-phagocytized conidia. Then the cells were fixed in 0.5% paraformaldehyde. Fluorescence was observed with a [fluorescencemicroscope](#). Phagocytic activity was calculated as a phagocytic index (PI) using the following formula:
$$\text{PI} = \frac{\text{Number of intracellular } T. \text{ marneffei conidia}}{\text{Number of macrophages containing } T. \text{ marneffei conidia}}$$

T. marneffei killing assay

The *T. marneffei* killing was evaluated by colony-forming unit (CFU) measurement. Pretreat LPS-activated macrophages with 20μM nor-NOHA for 2h. Then, the cells were co-cultured with *T. marneffei* conidia for 24h (MOI=1). After 24h of incubation, the supernatant was collected. Sterile water was added to each well and the plate was shaken adequately. Combine well contents into the supernatant. Centrifugation, abandonment of supernatant, suspension of sediment with sterile water. Then the fungi suspension was serially diluted in sterile water, and eventually plated (quintuplicate samples) on solid YPD agar plates.

Statistical analysis

Statistical analysis was performed with GraphPad Software 7.01. Unpaired Student's *t* test and one-way ANOVA were used. Values of *p* < 0.05 were considered to be significant, and data were shown as

mean±SD.

Abbreviations

T. marneffe: *Talaromyces marneffe*; Arg:Arginase; Arg1:Arginase-1; iNOS:Inducible Nitric Oxide Synthase; NO:Nitric Oxide; LPS:Lipopolysaccharides; IFN-γ:Interferon-γ; TNF-α:Tumor Necrosis Factor-α; nor-NOHA:N^ω-hydroxy-nor-arginine; MOI:Multiplicity of Infection; PDA:Potato Dextrose Agar Medium; YPD:Yeast Extract Peptone Dextrose Medium; SAMDC:S-adenosylmethionine decarboxylase.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. As to the raw data, please refer to the Additional file1.

Competing Interest

No conflict of interest to declare.

Funding

This work was supported by the National Natural Science Foundation of China (81760564). Funding bodies have no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Authors' contributions

DL and LS conceived and designed the experiments. LS, DY and RC performed the experiments. LS wrote the manuscript. LS, DY and RC analyzed the data. DL for critical reading and editing of the manuscript. DL and LS contributed reagents, materials and technical support. All authors reviewed the manuscript. All authors read and approved the final manuscript.

Acknowledgments

The authors extend their gratitude to Hong-lei Guo for invaluable technical assistance.

References

1. Cao C, Liang L, Wang W, Luo H, Huang S, Liu D, Xu J, Henk DA, Fisher MC. Common reservoirs for *Penicillium marneffei* infection in humans and rodents, China. *Emerg Infect Dis*. 2011;17:209–14.
2. Hamilton AJ, Jeavons L, Youngchim S, Vanittanakom N, Hay RJ. Sialic acid-dependent recognition of laminin by *Penicillium marneffei* conidia. *Infect Immun*. 1998;66:6024–6.
3. Patel U, Rajasingh S, Samanta S, Cao T, Dawn B, Rajasingh J. Macrophage polarization in response to epigenetic modifiers during infection and inflammation. *Drug discovery today*. 2017;22:186–93.
4. Shatanawi A, Romero MJ, Iddings JA, Chandra S, Umapathy NS, Verin AD, Caldwell RB, Caldwell RW. Angiotensin II-induced vascular endothelial dysfunction through RhoA/Rho kinase/p38 mitogen-activated protein kinase/arginase pathway. *American journal of physiology Cell physiology*. 2011;300:C1181–92.
5. Wagener J, MacCallum DM, Brown GD, Gow NAR, Doering TL. *Candida albicans* Chitin Increases Arginase-1 Activity in Human Macrophages, with an Impact on Macrophage Antimicrobial Functions. *mBio* 2017; 8.
6. Rópolo AAS, Touz MC. A Lesson in Survival, by *Giardia lamblia*. *The Scientific World JOURNAL*. 2010;10:2019–31.
7. Vincendeau P, Gobert AP, Daulouede S, Moynet D, Mossalayi MD. Arginases in parasitic diseases. *Trends Parasitol*. 2003;19:9–12.
8. Carl H, June JA, Bluestone, Nadler LM, Thompson CB. The B7 and CD28 receptor families. *Immunol Today*. 1994;15:321–31.
9. Taylor ME. Structure and function of the macrophage mannose receptor. *Results Probl Cell Differ*. 2001;33:105–21.
10. Rajaram MVS, Arnett E, Azad AK, Guirado E, Ni B, Gerberick AD, He LZ, Keler T, Thomas LJ, Lafuse WP, et al. *M. tuberculosis*-Initiated Human Mannose Receptor Signaling Regulates Macrophage Recognition and Vesicle Trafficking by FcRgamma-Chain, Grb2, and SHP-1. *Cell reports*. 2017;21:126–40.
11. Song EH, Osanya AO, Petersen CA, Poh NLB. Synthesis of multivalent tuberculosis and *Leishmania*-associated capping carbohydrates reveals structure-dependent responses allowing immune evasion. *J Am Chem Soc*. 2010;132:126–40.
12. Ranasinghe C, Trivedi S, Wijesundara DK, Jackson RJ. IL-4 and IL-13 receptors: Roles in immunity and powerful vaccine adjuvants. *Cytokine Growth Factor Rev*. 2014;25:437–42.
13. Pernow J, Kiss A, Tratsiakovich Y, Climent B. Tissue-specific up-regulation of arginase I and II induced by p38 MAPK mediates endothelial dysfunction in type 1 diabetes mellitus. *Br J Pharmacol*.

2015;172:4684–98.

14. Jung C, Gonon AT, Sjoquist PO, Lundberg JO, Pernow J. Arginase inhibition mediates cardioprotection during ischaemia-reperfusion. *Cardiovascular research*. 2010;85:147–54.
15. Miret JJ, Kirschmeier P, Koyama S, Zhu M, Li YY, Naito Y, Wu M, Malladi VS, Huang W, Walker W, et al. Suppression of Myeloid Cell Arginase Activity leads to Therapeutic Response in a NSCLC Mouse Model by Activating Anti-Tumor Immunity. *Journal for ImmunoTherapy of Cancer* 2019; 7.
16. Horowitz S, Binion DG, Nelson VM, Kanaa Y, Javadi P, Lazarova Z, Andrekopoulos C, Kalyanaraman B, Otterson MF, Rafiee P. Increased arginase activity and endothelial dysfunction in human inflammatory bowel disease. *American journal of physiology Gastrointestinal liver physiology*. 2007;292:G1323–36.
17. Kovamees O, Shemyakin A, Pernow J. Effect of arginase inhibition on ischemia-reperfusion injury in patients with coronary artery disease with and without diabetes mellitus. *PloS one*. 2014;9:e103260.
18. QuitterF, FigullaHR, Ferrari. M. Increased arginase levels in heart failure represent a therapeutic target to rescue microvascular perfusion. *Clin Hemorheol Microcirc*. 2013;54:75–85.
19. Kovamees O, Shemyakin A, Eriksson M, Angelin B, Pernow J. Arginase inhibition improves endothelial function in patients with familial hypercholesterolaemia irrespective of their cholesterol levels. *Journal of internal medicine*. 2016;279:477–84.
20. Holowatz LA, Thompson CS, Kenney WL. Acute ascorbate supplementation alone or combined with arginase inhibition augments reflex cutaneous vasodilation in aged human skin. *American journal of physiology Heart circulatory physiology*. 2006;291:H2965–70.
21. Pudlo M, Demougeot C, Girard-Thernier C. Arginase Inhibitors: A Rational Approach Over One Century. *Medicinal research reviews*. 2017;37:475–513.
22. Iniesta V, Gómez-Nieto LC, Corraliza I. The inhibition of arginase by N(omega)-hydroxy-L-arginine controls the growth of *Leishmania* inside macrophages. *J Exp Med*. 2001;193:777–84.
23. McGee DJ, Radcliff FJ, Mendz GL, Ferrero RL, Mobley HLT. *Helicobacter pylori* rocF is required for arginase activity and acid protection in vitro but is not essential for colonization of mice or for urease activity. *J Bacteriol*. 1999;181:7314–22.
24. Zabaleta J, McGee DJ, Zea AH, Hernández CP, Rodriguez PC, Sierra RA, Correa P, Ochoa AC. *Helicobacter pylori* arginase inhibits T cell proliferation and reduces the expression of the TCR zeta-chain (CD3zeta). *J Immunol*. 2004;173:586–93.
25. Ghosh S, Navarathna DH, Roberts DD, Cooper JT, Atkin AL, Petro TM, Nickerson KW. Arginine-induced germ tube formation in *Candida albicans* is essential for escape from murine macrophage line RAW 264.7. *Infect Immun* 2009; 77:1596–1605.
26. Zuber S, Hynes MJ, Andrianopoulos A. G-protein signaling mediates asexual development at 25 degrees C but has no effect on yeast-like growth at 37 degrees C in the dimorphic fungus *Penicillium mameffei*. *Eukaryot Cell*. 2002;1:440–7.
27. Iniesta V, Carcelen J, Molano I, Peixoto PMV, Redondo E, Parra P, Mangas M, Monroy I, Campo ML, Nieto CG, et al. Arginase I Induction during *Leishmania major* Infection Mediates the Development of

- Disease. Infect Immun. 2005;73:6085–90.
28. Hamana K, Matsuzaki S. Polyamines as a chemotaxonomic marker in bacterial systematics. Cirt Rev Microbiol. 1992;18:261–83.
 29. Gevrekci A. The roles of polyamines in microorganisms. World Journal of Microbiology and Biotechnology 2017; 33.
 30. Kummasook A, Cooper CR, Sakamoto A, Terui Y, Kashiwagi K, Vanittanakom N. Spermidine is required for morphogenesis in the human pathogenic fungus, *Penicillium marneffe*i. Fungal Genet Biol. 2013;58–59:25–32.
 31. Chan JFW, Chan TSY, Gill H, Lam FYF, Trendell-Smith NJ, Sridhar S, Tse H, Lau SKP, Hung IFN, Yuen K-Y, et al. Disseminated Infections with *Talaromyces marneffe*i in Non-AIDS Patients Given Monoclonal Antibodies against CD20 and Kinase Inhibitors. Emerg Infect Dis. 2015;21:1101–6.
 32. Pruetpongpun N, Khawcharoenporn T, Damronglerd P, Suthiwartnarueput W, Apisarnthanarak A, Rujanavej S, Suwantararat N. Disseminated *Talaromyces marneffe*i and *Mycobacterium abscessus* in a Patient With Anti-Interferon- γ Autoantibodies. Open Forum Infectious Diseases. 2016;3:ofw093.
 33. Zhang N, Deng J, Wu F, Lu X, Huang L, Zhao M. Expression of arginase I and inducible nitric oxide synthase in the peripheral blood and lymph nodes of HIV positive patients. Mol Med Rep. 2016;13:731–43.

Figures

Figure 1

T. marneffei affected the transcriptional expression of iNOS and Arg1 in macrophages. (A, B) Relative mRNA expression of iNOS and Arg1 in macrophages co-cultured with TM conidia for 24h, 48h and 72h (MOI=10), Mφ+TM vs Mφ, *P<0.05, **P<0.01; Mφ+TM vs Mφ+TM, ##P<0.01; (C, D) Relative mRNA expression of iNOS and Arg1 mRNA in LPS-activated macrophages co-cultured with TM conidia for 24h, 48h and 72h (MOI=10), Mφ+LPS vs Mφ, *P<0.05, **P<0.01; Mφ+LPS+TM vs Mφ+LPS, \$\$P<0.01; Mφ+LPS+TM vs Mφ+LPS+TM, ##P<0.01

Figure 2

T. marneffei affected the protein expression of iNOS and Arg1 in macrophages. (A-C) The iNOS protein expression in macrophages/LPS-activated macrophages co-cultured with TM conidia for 24h, 48h and 72h respectively (MOI=10) (D-E) The Arg1 protein expression in macrophages/LPS-activated macrophages co-cultured with TM conidia for 24h, 48h and 72h respectively (MOI=10), * $P \leq 0.05$, ** $P \leq 0.01$, ns: no significant.

Figure 3

T. marneffei affected the arginase activity and NO production in macrophages. (A, B) The arginase activity and NO production in macrophages co-cultured with TM conidia for 24h, 48h and 72h (MOI=10), Mφ+TM vs Mφ, *P<0.05, **P<0.01; Mφ+TM vs Mφ+TM, ##P<0.01 (C, D) The arginase activity and NO production in LPS-activated macrophages co-cultured with TM conidia for 24h, 48h and 72h (MOI=10), Mφ+LPS+TM vs Mφ+LPS, *P<0.05, **P<0.01; Mφ+LPS+TM vs Mφ+LPS+TM, #P<0.05, ##P<0.01

Figure 4

T. marneffei affected the macrophage polarization. (A-D) Relative mRNA expression of TNF-α, IL-1β, IL-10 and CD301 in macrophages co-cultured with TM conidia for 24h, 48h and 72h (MOI=10), Mφ+TM vs Mφ, ** $P \leq 0.01$; Mφ+TM vs Mφ+TM, # $P \leq 0.05$, ## $P \leq 0.01$. (E-H) Relative mRNA expression of TNF-α, IL-1β, IL-10 and CD301 mRNA in LPS-activated macrophages co-cultured with TM conidia for 24h, 48h and 72h

(MOI=10), M ϕ +LPS vs M ϕ , *P $\leq$ 0.05, **P $\leq$ 0.01; M ϕ +LPS+TM vs M ϕ +LPS, \$P $\leq$ 0.05, \$\$P $\leq$ 0.01; M ϕ +LPS+TM vs M ϕ +LPS+TM, #P $\leq$ 0.05, ##P $\leq$ 0.01

Figure 5

T. marneffei affected the protein expression of CD86, IL-4R and CD206 in macrophages. (A-C) The CD86 protein expression in macrophages/LPS-activated macrophages co-cultured with TM conidia for 24h, 48h and 72h respectively (MOI=10) (D) The IL-4R protein expression in macrophages/LPS-activated macrophages co-cultured with TM conidia for 24h, 48h and 72h respectively (MOI=10) (E) The fluorescence intensities of CD206 in macrophages/LPS-activated macrophages co-cultured with TM conidia for 72h (MOI=10), **P $\leq$ 0.01, ns: no significant.

Figure 6

Effect of nor-NOHA on the arginine metabolism pathway in co-culture system. (A, B) The Arg1 and iNOS protein expressions in LPS-activated macrophages pretreated with/without 20 μ M nor-NOHA before being co-cultured with TM conidia for 24h (MOI=10) (C, D) The arginase activity and NO production in LPS-activated macrophages pretreated with/without 20 μ M nor-NOHA before being co-cultured with TM conidia for 24h (MOI=10), nor-Neg vs Ctrl, #P<0.05, ##P<0.01, ns: no significant; nor-Posi vs nor-Neg, *P<0.05, **P<0.01.

Figure 7

Nor-NOHA promoted the antimicrobial function of macrophages. (A) Stained TM conidia with Calcofluor white and 10% KOH. LPS-activated macrophages pretreated with/without nor-NOHA for 2h before being co-cultured with stained TM conidia (MOI=10). The bright blue TM conidia in nor-Posi group was more than that in nor-Neg group under the fluorescence microscope. The phagocytic index of macrophages was calculated, nor-Posi vs nor-Neg, *P<0.05. (B-E) TM conidia treated with/without 20μM nor-NOHA for 2h; LPS-activated macrophages pretreated with/without 20μM nor-NOHA for 2h before being co-cultured with TM conidia for 24h (MOI=1), CFU was calculated by plate colony-counting method, nor-Posi vs nor-Neg, *P<0.05; nor-Neg vs TM, **P<0.01; TM-nor vs TM, *P<0.05;

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Originalfigure.pptx](#)