A Reliable Murine Model of Disseminated Infection Induced by *Talaromyces marneffei*

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Abstract Talaromycosis (penicilliosis) caused by *Talaromyces marneffei* is one of the most important opportunistic infection diseases in tropical countries of South and Southeast Asia. Most infections occurred in individuals with human immunodeficiency virus (HIV) and the primarily reason for the increase in the number of the cases is HIV pandemic. The pathogenesis of *T. marneffei* infection is unclear. There is still no ideal animal model for studying talaromycosis. In this study, we developed a stable, safe and maneuverable murine model that mimics human *T. marneffei* disseminated infection using *T. marneffei* yeast intraperitoneal injected to BALB/c nude mice. We successfully observed symptoms similar to those seen in clinical patients in this murine model, including skin lesions, hepatosplenomegaly, pulmonary infection and mesenteric lesions. We further studied the pathological changes of various tissues and organs in the infected animals to help better understand the severity of the infection. This model may provide a good tool for studying disseminated infection induced by *T. marneffei*.

Keywords *Talaromyces marneffei* · Animal model · Murine · Talaromycosis

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Introduction

Talaromyces marneffei (T. marneffei), formerly named Penicillium marneffei [1], is a thermally dimorphic fungus that causes lethal mycosis called talaromycosis (penicilliosis) in patients infected with human immunodeficiency virus (HIV) [2]. T. marneffei is the third most common opportunistic infection in HIV-infected patients in certain parts of Southeast Asia countries including Thailand, Vietnam and Southern China following extrapulmonary tuberculosis and cryptococcosis or pneumocystis [3]. T. marneffei can cause local infections and fatal disseminated infections. Disseminated infections were diagnosed in 86% of cases reported in the literature [4]. The mortality rate of patients with disseminated T. marneffei infection range from 10–30%, and even up to 97% if therapy is delayed [5–8]. As both basic and clinical knowledge about disseminated talaromycosis is limited, laboratory models of the disease are needed.

Murine models have predominated for most investigators over the years regarding that mice and humans have similarities in organ systems, biochemistries, pathologies [9]. Several murine models have been developed to study talaromycosis. Injection of T. marneffei yeast cells suspension into the lateral tail vein of mice had been used to assess the virulence of different strains [10]. A pulmonary infection murine model was created by intratracheal instillation of T. marneffei [11], whereas Liu et al. infected mice by using a nebulizer to deliver T. marneffei conidia to the lungs of BALB/c nude mice housed in exposure chamber [12]. Though these studies provided some new insights into the pathogenesis of talaromycosis, the methodologies are time-consuming or needing special equipment. A easy to operate animal model that can precisely mimic human T. marneffei infection is critical for study this disease and for drug screening and evaluation.

Materials and Methods

Animals and Ethics Statement

Congenitally athymic female BALB/c nude mice, weighing 18-21 g, were purchased from the experiment animal center of the Kunming Medical University and were used in this experiment at the age of 6–8 weeks. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of the Kunming Medical University. All mice were housed in a pathogen-free environment and received sterilized food and water at the Laboratory Animal Centre for Biomedical Sciences of the Kunming Medical University.

Microorganisms and Culture Conditions

T. marneffei strain ATCC18224 was purchased from American Type Culture Collection. The mycelial colony was cultured in brain heart infusion (BHI) plates incubated at 37 °C for 72 h following repeated subculturing until the yeast cells appeared. The newly grown yeast colonies were transferred to an agitated flask (150 r.p.m) containing BHI broth and incubated at 37 oC for 72 h. Yeast were harvested by centrifuging at 3000 g for 10 min, the resulting fungal suspensions were adjusted to the required concentrations using a hemocytometer.

The Infection Process and Survival Assay

Ten mice received solution from 0.1 ml of suspension containing $5 \times 10^8$ yeast/ml. Ten mice received solution from 0.1 ml of suspension containing $5 \times 10^7$ yeast/ml. Ten mice received solution from 0.1 ml of suspension containing $5 \times 10^6$ yeast/ml. Ten mice received solution from 0.1 ml of suspension containing $5 \times 10^7$ yeast/ml and those mice took amphotericin B (AMB) 5.0 mg/kg for treatment via intraperitoneal injection once daily on 8, 9, 10 and 14 days post-infection. Ten mice received 0.1 ml PBS as control. Those fifty mice were used for survival assay and body weight monitoring.

Histopathological Examination

Another eight mice, six mice received solution from 0.1 ml of a suspension containing $5 \times 10^7$ yeast/ml, and the other two euthanized before injection as control. On day 6, 12, 18 randomly chose two mice were used for collection of organ samples after euthanasia. We performed histopathological examination of lung, liver, spleen, kidney tissues of control,
6 days, 12 days euthanized mice and performed histopathological examination of lung, liver, spleen, kidney, skin, bone marrow, mesenteric nodules, rectum, small intestine tissues, brain and skeletal muscle tissues of 18 days euthanized mice. For histopathological assay, half of each organ was fixed in 4% neutral buffered formalin, processed, and embedded in paraﬃn. Tissue sections were stained with hematoxylin and eosin (H&E) and Periodic Acid-Silver-Meth-enamine (PASM) stain.

Statistical Analysis

Graphs were plotted by using GraphPad Prism 5 software (GraphPad Software, La Jolla, CA). Comparisons between groups were tested using independent samples t-test. Survival data were analyzed by means of log-rank comparisons of Kaplan–Meier survival curves.

Results

Establishment of the T. marneffei Infection Mouse Model

All the negative control group mice and 5 × 10^7 yeast/ml + AMB group mice were alive on the fortieth day after the inoculation. In the 5 × 10^8 yeast/ml group, the time of death was between 8 and 16 days. In the 5 × 10^7 yeast/ml group, 7 mice died between 20 and 22 days and the remaining 3 mice died on day 24, 25, 26 each. In the 5 × 10^6 yeast/ml group, the first death occurred on day 34 and six mice were still alive by day 40 (Fig. 1A). The body weight of the infected mice was signiﬁcantly reduced compared to that of the negative controls. Negative control group, the 5 × 10^7 yeast/ml group and the 5 × 10^7 yeast/ml + AMB group mice were monitored daily for body weight until day 19. Along with the gradual reduction of body weight of the 5 × 10^7 yeast/ml group, we observed a gradual increase of body weight in the PBS injected group and the 5 × 10^7 yeast/ml + AMB group mice. On Day 19 after injection, the mice in the 5 × 10^7 yeast/ml group lost 15.34% of their body weight compared with the body weight before injection; the average body weight of the infected group was 74.9% of that of the negative control group (independent samples t-test: t = −15.880, P < 0.001) and 80.5% of that of the 5 × 10^7 yeast/ml + AMB group (independent samples t-test: t = −10.665, P < 0.001) (Fig. 2B).

Gross Lesions and Autopsy Changes in BALB/c Nude Mice After Infection with T. marneffei

Mice in T. marneffei infected group developed skin lesions with central pitted papules (Fig. 2A-C) and diffuse scattered nodular lesions on the back (Fig. 2D). Autopsy examination of the infected mice showed hepatomegaly (Fig. 2E), splenomegaly (Fig. 2F), lung injury (Fig. 2G) and mesenteric nodules (Fig. 2H), and the fungus culture with mesenteric nodule showed T. marneffei (Supplementary Fig. 1). Mice skin lesions with central pitted papules are very similar to those in T. marneffei infected patients (Fig. 2I) and diffuse scattered nodular lesions are very similar to the lesions on the back of T. marneffei infected patients (Fig. 2J).

Histopathological Findings

The damage of each organ and the yeast cells were further analyzed by H&E and PASM staining respectively (Fig. 3). Tissue section of the lung showing diffuse thickening of interalveolar septa, destruction of the alveolar and tracheobronchial structure, blood exudation, inﬁltration of inﬂammatory cells consisting mainly of leukocyte and mononuclear (Fig. 3A-1), and macrophages and multinucleated giant cells loaded with large numbers of yeast cells (Fig. 3A-2, green arrow). Tissue section of the skin showing damage to skin structure, partial tissue defect of epidermis and dermal, epidermis trochanterecellus disappear at the site of fungal infection, structure boundary between dermis and epidermis is not clear, inﬁltration of inﬂammatory cells consisting mainly of leukocyte (Fig. 3B-1), and fungal yeast was found in the epidermis, dermis and subcutaneous tissues (Fig. 3B-2). The structure of the rectum is generally normal (Fig. 3C-1), but a small amount of fungal yeast
can be seen scattered in the mucosal layer (Fig. 3C-2). Focal lesions are seen in the small intestine with deformed intestinal villus, infiltration of inflammatory cells, structures of serosal layer destroys and connects nodular lesions (Fig. 3D-1), and fungal yeast was found in the lamina propria and nodular lesions (Fig. 3D-2). Throughout the spleen there were extensive structural damages often with structure disappeared of white pulp, medullary substance and trabeculae lienis (Fig. 3E-1) as well as numerous macrophages loaded with yeast cells (Fig. 3E-2). Liver section primarily characterized by granuloma formation, leukocyte infiltration (Fig. 3F-1) and macrophages containing many yeast-form cells of T. marneffei. AMB group were inoculated with 0.1 ml of suspension containing $5 \times 10^7$ yeast/ml and took amphotericin B 5.0 mg/kg for treatment. $5 \times 10^5$ yeast/ml group were inoculated with 0.1 ml of suspension containing $5 \times 10^5$ yeast/ml. Negative control group, $5 \times 10^7$ yeast/ml and $5 \times 10^5$ yeast/ml + AMB group mice were monitored daily for body weight until there were dead mice.

Fig. 1 Comparison of the survival rate (A) and body weights (B) between BALB/c nude mice infected with T. marneffei and mice inoculated with PBS. Negative control animals were inoculated with PBS. $5 \times 10^5$ yeast/ml group were inoculated with 0.1 ml of suspension containing $5 \times 10^5$ yeast/ml. $5 \times 10^7$ yeast/ml group were inoculated with 0.1 ml of suspension containing $5 \times 10^7$ yeast/ml. $5 \times 10^5$ yeast/ml + AMB group were inoculated with 0.1 ml of suspension containing $5 \times 10^5$ yeast/ml and took amphotericin B 5.0 mg/kg for treatment. $5 \times 10^5$ yeast/ml group were inoculated with 0.1 ml of suspension containing $5 \times 10^5$ yeast/ml. Negative control group, $5 \times 10^7$ yeast/ml group and $5 \times 10^5$ yeast/ml + AMB group mice were monitored daily for body weight until there were dead mice.

Fig. 2 Anatomical manifestations of T. marneffei infected mice and corresponding skin lesions picture of T. marneffei infected patients. A-C Skin lesions in different parts of BALB/c nude mice infected with T. marneffei. D: Nodular lesion of BALB/c nude mice infected with T. marneffei. E: Hepatomegaly of BALB/c nude mice infected with T. marneffei. F: Spleomegaly of BALB/c nude mice infected with T. marneffei. G: Pulmonary lesions of BALB/c nude mice infected with T. marneffei. H: Mesenteric lesion of BALB/c nude mice infected with T. marneffei. I: Facial skin lesions of patients with talaromycosis. J: Back lesions in patients with talaromycosis.
Fig. 3 HE (n-1) and PASM (n-2) analyses of BALB/c nude mice infected with T. marneffei. Fungal yeasts were observed in the lung (A), shin (B), rectum (C), small intestine (D), mesenteric nodules (D), spleen (E), liver (F), kidney (G), bone marrow tissues (H) of the infected mice. Magnification: The lower right corner × 4, scale bars: 2000 μm. The top left corner × 400, scale bars: 20 μm.
Fig. 3 continued
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marneffei (Fig. 3F-2). In the kidneys were characterized by scattered lesions (Fig. 3G-1) and yeast cells (Fig. 3G-2) in the glomeruli and cortical interstitium. Fungal yeast is also found in the femoral bone marrow (Fig. 3H-1). No pathological changes and fungal yeast were observed in brain tissue and skeletal muscle tissue (Supplementary Fig. 2).

We observed the liver, spleen, lung and kidney histopathology changes using PASM at four time points before the infected mice sacrificed: before the infection, the 6th day after infection, the 12th day after infection and the 18th day after infection. No fungal yeast infiltration was observed in the organs of blank control mice(Fig. 4A-D). On day 6, no obvious fungal yeast infiltration was observed in the organs(Fig. 4E-H). Fungal yeast was found in the liver, spleen, lung and kidney tissues on day 12(Fig. 4I-L) and 18(Fig. 4M-P). Compared with day 12, the number and size of fungal infiltrating area were larger. (The green arrow indicates the fungal yeast.)

Discussion

In this study, we established a new experimental murine model of disseminated T. marneffei infection closely mimic the clinic feature of T. marneffei infection observed in humans. The most common clinical features observed in patients with T. marneffei include fever (98.0%), anemia (74.8%), and weight loss (71.6%) [13]. These three symptoms are common non-specific symptoms of infectious diseases. Dissemination of the disease is characterized by skin lesions (69.7%-85%), such as papules with central necrosis [2, 13]. This characteristic lesion can be a single or first-episode symptom of talaromycosis and has a strong suggestive role in the diagnosis of this disease [14]. It is therefore important to observe this particular lesion in a mouse model. The gross appearance of skin lesions in our model is consistent with that of human skin lesions in infected patients. Pulmonary infiltrates, lymphadenopathy, splenomegaly, and hepatomegaly were also noted in many patients [13]. Lesions in these organs were also found in the anatomy of our model group. Previous studies had reported diffuse infiltration of macrophages engorged with proliferating yeast cells in tissues [2, 15] and a variety of organs including the skin, bone marrow, lymph nodes, liver, lung, bone, spleen, nasopharynx, bowel, kidney, pericardium, meninges [13]. We observed fungal yeasts in the skin, bone marrow, mesenteric nodules, liver, lung, spleen, kidney, rectum, small intestine tissues, but not in the brain and skeletal muscle. Except for the meninges, our model infection organs were almost identical to the infected organs of patients reported in the literature. Therefore, our murine model successfully mimicked deep-seated visceral fungal infection, and it was the first time that our murine model had skin lesions similar to those found in human patients.

Our modeling method has some advantages over the modeling method in the previous studies [10–12]). The first advantage is that it is easy to operate and no special equipment is needed. Recently, Liu et al. had developed a murine pulmonary T. marneffei infection model, and they used BALB/c nude mice with a nebulizer to deliver T. marneffei conidia to the lungs with mice housed in exposure chamber [12]. The procedure needs a nebulizer driven by compressed air to generate a suspension containing conidia to aerosols and then input into a multi-animal exposure chamber. After passing through the exposure chamber the air is needed to filter before being sent into the exhaust system. The generated aerosol was connected to the exposure chamber, which was connected to an air filter through another tube to prevent spores from contaminating the environment. They first constructed a murine pulmonary model that would mimic human talaromycosis utilizing inhalation exposure [12]. In their study, not only special experimental equipment but also air pollution and safety issues need to be considered. In another study, Sun et al. injected T. marneffei yeast suspension the lateral tail vein of mice to establish the infection [10]. However, in operation, the fungal yeast easily agglomerates or precipitates, thus increasing the risk of pulmonary embolism in mice. Kudenken et al. established T. marneffei infection in mice by intratracheal instillation, which is also a complex procedure and time-consuming [11]. Based on our experiments, the recommended dose is 0.1 ml of a suspension containing 5 × 10⁷ yeast/ml. The second advantage is that no big difference in the total survival time of each T. marneffei infected mouse of the 5 × 10⁷ yeast/ml group, with the first death on day 20 and all death on day 26. However, in the inhaled mouse model [12], animals began to die on day 15 after infection and some were still 35% alive on day 40. In the tail vein injection mouse model [10],
animals began to die on day 8 after infection and all the mice died on day 18, animals of the intratracheal instillation mouse model began to die on day 33 after infection and all the mice died day 44 [11]. And the third advantage is that our mouse model was able to be treated with AMB which reduced weight loss in mice and extends survival with no dead mice by the end of our observation. The results of the treatment mice show that our model can be used for the development of therapeutic drugs.

In addition to mouse model, several other species models have been developed to research T. marneffei infection such as Galleria mellonella Larvae, Caenorhabditis elegan and zebrafish, which were showed their own unique advantages [16–21]. Huang et al. [16] showed that T. marneffei was able to infect the G. mellonella at both 25 and 37°C. But murine model can only study T. marneffei at 36.5°C in vivo. The G. mellonella model makes up for the deficiency of the murine model which cannot study T. marneffei at 25°C in vivo. Caenorhabditis elegan is another simple nematode infection model for T. marneffei, but the C. elegans were incubated at 25°C which caused T. marneffei hyphae form inside nematode worm. This is not consistent with the fact that T. marneffei pathogenic state in the human body is yeast form [18]. The zebrafish is a desirable host model for studying microbial infections because it combine the advantages of small size, optical transparency and suitability for genetic manipulation. At present, the application of zebrafish in T. marneffei infection mainly focused on the study of virulence and the effect of

Fig. 4 Dynamic PASM analyses of main organs in BALB/c nude mice infection with T. marneffei. PASM analyses of Liver (A), spleen (B), lung (C) and kidney (D) of blank control mice; PASM analyses of Liver (E), spleen (F), lung (G) and kidney (H) of T. marneffei infected mice on day 6; PASM analyses of Liver (I), spleen (J), lung (K) and kidney (L) of T. marneffei infected mice on day 12; PASM analyses of Liver (M), spleen (N), lung (H) and kidney (I) of T. marneffei infected mice on day 18, scale bars: 500 µm.
innate immunity on *T. marneffei* [19–21]. *G. mellonella, C. elegans* and zebrafish are easier to manipulate and more economical than murine. These three models are mainly used in the study of *T. marneffei* virulence and innate immunity on *T. marneffei*. However, because huge racial differences exist between invertebrates and humans, these three models also have limited value for mechanistic studies of *T. marneffei*-host adaptive immunity interactions and conclusions of those studies should be treated with caution.

Our model also has some limitations. Firstly, intraperitoneal injection is not a natural route of *T. marneffei* infection. Secondly, injection using *T. marneffei* yeast directly causes invasive infection. So this model cannot be used to study the mechanism of *T. marneffei* conidia colonization and invasion in vivo. BALB/c nude mice has no thymus and T lymphocyte defects so this model cannot be used to study the T cell immunity function in *T. marneffei*.

In conclusion, we successfully developed a stable, safe and maneuverable murine model of *T. marneffei* infection. The symptoms and diseased organs of this model are highly consistent with the disseminated *T. marneffei* infections in patients. This murine model may be used for understanding the pathogenesis of this disease.

**Author contributions** Conceived and designed the experiments: RW YL. Performed the experiments: JH JL HX JL ZL HZ. Analyzed the data: JH YK HL. Contributed reagents/materials/analysis tools: HL RW YL. Contributed to the writing of the manuscript: JH RW YL.

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**Declarations**

**Conflict of interest** The authors declare that they have no conflict of interest.

**Consent to publish** The authors affirm that human research participants provided informed consent for publication of the images in Fig. 2I, 2J. Patients signed informed consent regarding publishing their data and photographs.

**References**

1. Samson RA, Yilmaz N, Houbraken J, et al. Phylogeny and nomenclature of the genus Talaromyces and taxa accommodated in *Penicillium* subgenus Biverticillium. Stud Mycol. 2011;70:159–83. https://doi.org/10.3114/sim.2011.70.04.
2. Vanittanakom N, Cooper CR Jr, Fisher MC, et al. *Penicillium marneffei* infection and recent advances in the epidemiology and molecular biology aspects. Clin Microbiol Rev. 2006;19:95–110. https://doi.org/10.1128/cmr.19.1.95-110.2006.
3. Limper AH, Adenis A, Le T, et al. Fungal infections in HIV/AIDS. Lancet Infect Dis. 2017;17:e34-43. https://doi.org/10.1016/s1473-3099(17)30303-1.
4. Nittayananta W. *Penicilliosis marneffei*: another AIDS defining illness in Southeast Asia. Oral Dis. 1999;5:286–93. https://doi.org/10.1111/j.1601-0825.1999.tb00091.x.
5. Jiang J, Meng S, Huang S, et al. Effects of Talaromyces marneffei infection on mortality of HIV/AIDS patients in southern China: a retrospective cohort study. Clin Microbiol Infect: Off Publ Eur Soc Clin Microbiol Infect Dis. 2019;25:233–41. https://doi.org/10.1016/j.cmi.2018.04.018.
6. Hu Y, Zhang J, Li X, et al. *Penicillium marneffei* infection: an emerging disease in mainland China. Mycopathologia. 2013;175:57–67. https://doi.org/10.1007/s11046-012-9577-0.
7. Charityalertsak S, Suprapaturiyko S, Sirisanthana T, et al. A controlled trial of itraconazole as primary prophylaxis for systemic fungal infections in patients with advanced human immunodeficiency virus infection in Thailand. Clin Infect Dis: Off Publ Infect Dis Soc Am. 2002;34:277–84. https://doi.org/10.1086/338154.
8. Son VT, Khue PM, Strobel M. *Penicilliosis* and AIDS in Haiphong, Vietnam: evolution and predictive factors of death. Med et Maladies Infect. 2014;44:495–501. https://doi.org/10.1016/j.medmal.2014.09.008.
9. Waterston RH, Lindblad-Toh K, Birney E, et al. Initial sequencing and comparative analysis of the mouse genome. Nature. 2002;420:520–62. https://doi.org/10.1038/nature01262.
10. Sun J, Li X, Feng P, et al. RNAi-mediated silencing of fungal acuD gene attenuates the virulence of *Penicillium marneffei*. Med Mycol. 2014;52:167–78. https://doi.org/10.1093/mmy/myt006.
11. Kudeken N, Kawakami K, Kusano N, et al. Cell-mediated immunity in host resistance against infection caused by *Penicillium marneffei*. J Med Vet Mycol: Bi-monthly Pub Int Soc Human and Animal Mycol. 1996;34:371–8. https://doi.org/10.1080/02681219680006671.
12. Liu Y, Huang X, Yi X, et al. Detection of talaromyces marneffei from fresh tissue of an inhalational murine pulmonary model using nested PCR. PLoS ONE.
13. Duong TA. Infection due to Penicillium marneffei, an emerging pathogen: review of 155 reported cases. Clin Infect Dis: An Off Pub Infect Dis Soc Am. 1996;23:125–30. https://doi.org/10.1093/clinids/23.1.125.

14. Cao C, Xi L, Chaturvedi V. Talaromycosis (Penicilliosis) due to Talaromyces (Penicillium) marneffei: insights into the clinical trends of a major fungal disease 60 years after the discovery of the pathogen. Mycopathologia. 2019;184:709–20. https://doi.org/10.1007/s11046-019-00410-2.

15. Chan YF, Chow TC. Ultrastructural observations on Penicillium marneffei in natural human infection. Ultrastruct Pathol. 1990;14:439–52. https://doi.org/10.3109/01913129009007223.

16. Huang X, Li D, Xi L, et al. Galleria mellonella larvae as an infection model for Penicillium marneffei. Mycopathologia. 2015;180:159–64. https://doi.org/10.1007/s11046-015-9897-y.

17. Borman AM, Fraser M, Szekely A, et al. Rapid and robust identification of clinical isolates of Talaromyces marneffei based on MALDI-TOF mass spectrometry or dimorphism in Galleria mellonella. Med Mycol. 2019;57:969–75. https://doi.org/10.1093/mmy/myy162.

18. Huang X, Li D, Xi L, et al. Caenorhabditis elegans: a simple nematode infection model for Penicillium marneffei. PLoS ONE. 2014;9:e108764. https://doi.org/10.1371/journal.pone.0108764.

19. Cao C, Xi L, Chaturvedi V. Talaromycosis (Penicilliosis) due to Talaromyces (Penicillium) marneffei: insights into the clinical trends of a major fungal disease 60 years after the discovery of the pathogen. Mycopathologia. 2019;184:709–20. https://doi.org/10.1007/s11046-019-00410-2.

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