Perspective on animal models of dermatophytosis caused by *Trichophyton rubrum*

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In this issue of *Virulence*, Yoshikawa et al.1 described the role of the NLRP3 inflammasome in the response of macrophages to *Trichophyton rubrum* conidia, as well as the influence of interleukin-1 (IL-1) signaling in this interaction. On analyzing bone marrow-derived macrophages (BMDMs), the authors observed reduced IL-1β production in response to *T. rubrum* conidia in NLRP3−/−, ASC−/− and caspase-1/11−/− cells. They also found that IL-1 signaling was important for delaying hyphal development in fungi, thereby protecting macrophages from destruction by the fungus. These findings yielded new and important perspectives for better understanding of the immunological aspects of *T. rubrum* infection. Although the authors did not provide *in vitro* evidence regarding which molecular fungal structures are involved with NLRP3 inflammasome activation, the study provides a novel point of view about this axis of immune responses triggered by *T. rubrum* infection. They used intraperitoneal infection with *T. rubrum* conidia and reported fungal recovery from the liver for IL-1R−/− and wild-type (WT) mice, but they did not find a statistical significant difference between the groups at any of the time points evaluated. Lower levels of IL-17 were observed for the IL-1R−/− group, leading the authors to suggest the involvement of this pathway in the *in vivo* infection by *T. rubrum*. The main concern about this *in vivo* model is the route of infection, since the occurrence of *T. rubrum* in deep organs, such as the liver and spleen, is not common, and also because of the lack of histopathological analysis of fungal infection in the liver tissue. This concern culminates in the great question yet to be answered: are the *in vitro*, *ex vivo*, and *in vivo* models of dermatophytosis caused by *T. rubrum* able to mimic the inflammatory response provided by the human skin against this fungus?

*Trichophyton rubrum* belongs to dermatophytes, a group of filamentous fungi that is composed of 3 genera: *Epidermophyton*, *Microsporum*, and *Trichophyton*. These organisms are also classified according to habitat, into anthropophilic, zoophilic, and geophilic fungi,2 with *Trichophyton rubrum* being the most common etiologic agent of dermatophytosis.3,4 Dermatophytes are known as keratinophilic fungi because of their predilection to infect keratinized tissues, e.g., skin, nail, hair, and horns,2 implying that these tissues should be prioritized for the study of the host response. The clinical aspects of lesions are diversified and are a result of the delicate balance between the destruction of the keratin and the inflammatory response, which varies according to the etiologic agent and geographical localization.5,6

Morphologically, *T. rubrum* may present hyaline hyphae, microconidia, and macroconidia in addition to chlamydoid conidia and arthroconidia.2,7 Moreover, all these components may be involved in the infection, and arthroconidia represent the main infective structure for humans. This unique characteristic of *T. rubrum*, associated with the fact that it is an anthropophilic fungus, is the first challenge faced by a researcher who wants to study the disease in an animal model. It is difficult to determine the number of viable structures for infection (mainly arthroconidia); therefore, most studies use microconidia as a standard fungal structure for infection of animals.8,9 All these issues make the study of *T. rubrum* difficult and therefore only a small number of researchers have focused on the unknown aspects of fungus-host interactions.

Most animal models of dermatophytosis were obtained using zoophilic fungi, such as *T. mentagrophytes* or *Microsporum canis*, and guinea pigs are often the animal chosen.10,11 Animal models using *T. rubrum* as the etiologic agent are scarce, and therefore little is known about the fungus-host interactions. Although the guinea pig model has many characteristics in common with human skin, work with guinea pigs has a few drawbacks, such as the difficulty in manipulating the animals and the necessity of a large space for cages (mostly because of the large size of the animal), as well as the most important drawback: the lack of knockout (KO) animals. In contrast, mice are easy to manipulate, are less time-consuming to study, and can be placed in small cages. Notwithstanding the fact that experimental infections on the backs of mice may lead to spontaneous healing in about 4 weeks or more after infection, mice are still a good choice as an animal model. Furthermore, it is easier to obtain KO animals with a mouse model. In this context, different studies support the use of mice for experimental dermatophytosis.8,12,13 A previous study used *T. quinckeanum* to infect BALB/c mice, demonstrating pathological alterations and adherence of microconidia to keratinocytes within 4 hours of infection. In addition, early infiltration of
neutrophils and formation of a mycelial mass (scutulum) in the epidermis were observed. Venturini et al. reported an invasive model of dermatophytosis involving Trichophyton in Swiss mice. On performing subcutaneous injection into the footpad, the authors observed rapid spread of fungal cells to the popliteal lymph nodes, spleen, liver, and kidneys. In addition, the study showed a Th1-polarized immune response. Using a different approach, Nakamura et al. established a mouse model of Trichophyton-induced contact hypersensitivity by using an extract from Trichophyton and showed that mice mounted different inflammatory responses according to the immunological background. C57BL/6 mice presented not only Th1 but also Th17 cells; however, in BALB/c mice, thymic stromal lymphopoietin (TSLP) and IL-4 were enhanced after challenge. Th17 cells; however, in BALB/c mice, thymic stromal lymphopoietin (TSLP) and IL-4 were enhanced after challenge.

Macrofage cells

Chinese hamster ovary epithelial (CHO) cells

Nail powder from healthy toenails

Primary macrophage cells

PBMC

Biopsy specimens from skin lesions

Table 1. In vitro and ex vivo models of infection with dermatophytes

| Model of study | Dermatophyte species | Outcome | Reference |
|---------------|----------------------|---------|-----------|
| O-ring fixed on distal fingernail fragments from adult volunteers | Microconidia of T. mentagrophytes | Nail fragment was surrounded by a fungal colony by day 5, and the colony reached the maximum size on day 15 after infection. The colony reached the ventral side of the nail on the 5th day, and the area inside the ring was filled on the 14th day. | Nakashima et al. |
| Chinese hamster ovary epithelial (CHO) cells | Microconidia of T. mentagrophytes | Fungal cells presented carbohydrate-specific adhesins on the microconidia surface that recognized mannose and galactose, which may be important for the adhesion and invasion of the fungus during infection. | Esquenazi et al. |
| CHO and macrophage cells | Microconidia of T. rubrum | T. rubrum expressed carbohydrate-specific adhesins on the microconidia surface that recognized mannose and galactose, which might be important for the adhesion and invasion of the fungus during infection. | Esquenazi et al. |
| Nail powder from healthy toenails | Microconidia of T. rubrum | Arthroconidia production in ground nails did not occur until the replacement of the atmosphere with 10% CO2 and took about 14 d to reach maximal levels. | Yezdanparast et al. |
| Macrophage cells | Microconidia of T. rubrum | Fungal cells induced the production of tumor necrosis factor α (TNF-α) and IL-10, but not IL-12 and nitric oxide. In addition, macrophage viability decreased after 8 h of conidia ingestion. | Campos et al. |
| Human epidermal keratinocytes (NHEKs) | T. mentagrophytes, T. rubrum, and T. tonsurans | NHEKs cocultured with T. mentagrophytes presented the highest levels of IL-8 and GRO-α compared to those from T. rubrum and T. tonsurans. | Tani et al. |
| Peripheral blood mononuclear cells (PBMC) | T. mentagrophytes or T. rubrum | Both CD4+ and CD8+ T cells possessed cytotoxic activity against both fungal species and may play a role in the host defense. | Waldman et al. |
| Primary macrophage cells | Microconidia of T. rubrum | T. rubrum conidia were killed through the production of reactive oxygen species. In addition, fungal cells were more efficiently engulfed and killed by macrophages from wild-type mice than by cells from IL-12−/− and IFN-γ−/− mice. | Baltazar et al. |
| PBMC | Microconidia of T. rubrum | T. rubrum conidia differentiated into hyphae, killing macrophages. But dendritic cells were able to kill T. rubrum. | Santiago et al. |
| Biopsy specimens from skin lesions | Tissues from localized (LD) and disseminated (DD) dermatophytes by T. rubrum | Reduced TLR4 expression, but not TLR2 expression, was detected in both DD and LD patients compared with the control group. | Oliveira et al. |
in vitro or ex vivo approaches, as detailed in Table 1. However, while these approaches are very useful for elucidating some aspects of the fungus-host interaction, important questions about the immune response are still unanswered. This information reinforces the need for the development and characterization of models by using anthropophilic fungi, in order to improve the understanding of fungus-host interactions. From this point of view, Baltazar et al.8 reported the successful use of mice as an animal model of dermatophytosis caused by T. rubrum. They showed that C57BL/6 WT mice presented high fungal burdens, mild dermatitis, and epidermal hyperplasia on the 7th day after infection. In addition, they reported the importance of the proinflammatory cytokines IL-12 and gamma interferon (IFN-γ) to control the infection, since IL-12 and IFN-γ/β were shown to control the infection, while IFN-γ/β−−/− mice showed higher fungal burdens on the skin and lower IL-1β levels than the WT group. After establishing the model of dermatophytosis by T. rubrum, Baltazar et al.15 and Gasparoto et al.16 reinforced the reproducibility of the model, even using animals with different backgrounds, i.e., C57BL/6 and BALB/c mice, respectively. Baltazar et al.15 treated T. rubrum infection with photodynamic therapy, demonstrating that diode emission at 630 nm and toluidine blue reduced the fungal burden in the skin compared to the level seen in the untreated control. In the same manner, Gasparoto et al.16 showed that 2-(benzylidenecamino)phenol (3A3), a new aldimine compound, was as efficient as itraconazole in reducing the fungal burden on the skin of mice, which is encouraging for future clinical investigations. Overall, significant efforts have been made toward a better understanding of the pathogenesis of dermatophytosis, but there are still a lot of gaps to be filled. For example, the models have shown that IFN-γ, IL-12, IL-1β, and IL-17 are important cytokines for controlling T. rubrum infection and that skin lesions of patients present lower expression of Toll-like receptor 4 (TLR4) than that seen in the control groups.1,17,18 Furthermore, both dendritic cells (DCs) and macrophages interact with T. rubrum conidia,8,19,18 but Santiago et al.18 demonstrated that only DCs inhibited T. rubrum growth and induced Th activation, suggesting that these cells play an important role in coordinating the development of the cellular immune response during T. rubrum infection. In conclusion, much work is still required to provide scientific information about dermatophyte infections to a level at least similar to that for other fungal infections caused by species that are easier to manipulate (e.g., Candida and Cryptococcus).19,20 The development of models specific for each dermatophyte species will provide valuable information regarding the dermatophyte-host relationship and will yield new perspectives to increase the understanding of its immunological and pathological aspects.

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No potential conflict of interest was disclosed.

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