Review

A Mouse Model of *Trichophyton* Inflammation Based on Trichophytin-induced Contact Hypersensitivity

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ABSTRACT

The prevalence of *Trichophyton*-induced superficial skin mycosis is very high among human patients. Dermatophytes generally infect the epidermis, especially the stratum corneum, forming scales, hyperkeratosis, and vesicles. The important roles played by the immune system in *Trichophyton* infection are detection of fungal invasion and elimination of fungi. These immune mechanisms are presumed to involve not only innate immunity but also acquired immunity. Therefore, there is a substantial need for studies on treatment methods based on new basic knowledge, and the elucidation of immunological mechanisms of *Trichophyton*-induced inflammatory reactions is especially important. However, since *Trichophyton* cannot colonize on the mouse skin, we tried to develop a model for *Trichophyton* inflammation induced by trichophytin extracted from *Trichophyton mentagrophytes* using a method based on contact hypersensitivity. Trichophytin is a crude extract that mainly contains fungal cell wall constituents including β-glucan and zymosan. In this model, TLR2, TLR4, and dectin-1 were highly expressed, and production of IL-17A and IL23 was observed. This indicates that we succeeded in inducing fungal-specific inflammation in the mice.

In this review, we introduce a mouse *Trichophyton* inflammation model developed to investigate the immunological mechanisms of *Trichophyton*-induced inflammatory reactions. In addition, we report results of evaluation of anti-inflammatory and anti-itching effects of anti-fungal agents using the inflammation model.

Key words: inflammation, pruritus, Trichophytin, *Trichophyton* inflammation model

Introduction

Superficial skin mycosis is an infectious disease mainly caused by dermatophytes, and its prevalence is very high in the Japanese population1,2. It often causes inflammation with itching3,4. Dermatophytes generally infect the epidermis, especially the stratum corneum, forming scales, hyperkeratosis, and vesicles. Among the dermatophytes, *Trichophyton* is known as the most frequent causative fungi in human beings.

The important roles played by the immune system in *Trichophyton* infection are detection of fungal invasion and elimination of fungi. These immune mechanisms are presumed to involve not only innate immunity but also acquired immunity. Peripheral blood mononuclear cells from patients with *Trichophyton*-induced inflammation showed potent proliferative ability in response to *Trichophyton* antigen and produced IFN-γ5. IFN-γ gene expression was found to be enhanced, and IFN-γ-positive CD4+ cells were present in the lesions6,7. Despite the necessity to elucidate the pathophysiology, including immune response, of superficial skin mycosis, there is little knowledge of the immunological mechanisms of *Trichophyton*-induced inflammatory reactions. An animal model that is useful for clarifying the pathological mechanisms is therefore necessary. Since *Trichophyton* cannot spontaneously colonize on the mouse skin, we tried to develop a model induced by trichophytin extracted from *Trichophyton mentagrophytes* using a method based on contact hypersensitivity (CHS)8. Trichophytin is a crude extract that mainly contains fungal cell wall constituents, including β-glucan and zymosan. It has been used to test for past infection of human skin by dermatophytes, and strongly positive tests are found in patients with kerion celsi and tinea barbae9.
In this model, TLR2, TLR4, and dectin-1 were highly expressed, and production of IL-17A and IL-23 was observed. That is, we succeeded in inducing fungal-specific inflammation in the mice. In this review, we will discuss findings obtained from the model and further show experimental results on pharmacokinetics/efficacy evaluation and scratching behavior using the mouse model.

**Trichophyton** inflammation model based on trichophythin-induced contact hypersensitivity

We established a *Trichophyton* inflammation model by applying a *Trichophyton* antigen, trichophythin (which is extracted from *T. mentagrophytes*), to mice. A mouse was sensitized with trichophythin transdermally on the back and then challenged on the ear. As a result, dominant swelling and inflammation of the challenged site were observed in the sensitized mice (Fig. 1a). Investigation of inflammatory cytokines and receptors of the innate immune system in the model showed that IFN-γ, IL-6, MIP-2, and IL-23 increased in the skin where inflammation was evoked. Likewise, expression of IFN-γ, IL-17A, TLR 4, TLR 2, and dectin-1 also increased in the draining lymph nodes (Fig. 1b).

The immune response in CHS is usually controlled by Th1-type immunity. The model indicated high production of IL-17A and IL-23, as well as IFN-γ, IL-6, and Mip-2, and expression of pattern recognition receptors, such as TLR-2, TLR-4, and dectin-1. The model, therefore, showed characteristics of both Th1-type and Th17-type immunity in spite of the inflammation being established based on the general CHS method. This means that the inflammation seen in this model can be induced by fungal components. Below are the findings of some investigations conducted using the model.

**Evaluation of anti-inflammatory agents**

Some traditional herbal medicines have anti-inflammatory effects. In particular, glycyrrhetic acid (GA), which is from plants of the genus *Glycyrrhiza* (liquorice), is known to have anti-inflammatory, anti-allergic, and anti-viral effects. GA down-regulates IL-8 production induced by *Trichophyton* infection in human epithelial cells. In addition, GA has anti-viral and anti-fungal effects. We therefore focused on the potential of GA as a remedy for fungal infection and investigated the effects of GA on *Trichophyton* inflammation by using this model.

GA treatment after evoking inflammation with trichophythin significantly suppressed swelling of the ear in a dose-dependent manner (Fig. 2a, b). Pathological findings showed that GA treatment suppressed spongiosis and decreased inflammatory cell infiltration to the dermis. Without treatment with GA, the mRNA expression levels of IFN-γ, IL-6, and MIP-2 in the ear after 24 h significantly increased. Meanwhile, GA treatment significantly suppressed the expression of all the above inflammatory markers, demonstrating that GA had anti-inflammatory effects against trichophythin-induced inflammation (Fig. 2c).

**Evaluation of itching in the model and efficacy of anti-fungal agents against pruritus**

Watanabe reported that 48% of superficial skin mycosis patients infected by *Trichophyton* experienced itching, and itching is reported to be correlated with skin inflammation. We observed that the mice treated with trichophythin exhibited scratching behavior similar to human patients infected with *Trichophyton*. Therefore, we measured scratching behavior of the trichophythin-inflamed mice by using an automatic counting device (MicroAct) up to 14 h after induction of inflammation. Injection of trichophytin into the back skin of mice significantly exacerbated the scratching behavior, which showed two peaks during the 14-h observation period (Fig. 3a, b).

Some anti-fungal agents are reported to have not only anti-fungal activities but also anti-inflammatory effects. For example, the imidazole-type ketoconazole (KCZ) can down-regulate both the degranulation of mast cells and the release of histamine. The thiocarbamate-type liranalactate (LNF) is known to inhibit the production of IL-8 in a test using the normal human epidermal keratinocytes (NHEK). The allylamine-type terbinafine (TBF) is known to inhibit the production of chemokines released from keratinocytes.

We then investigated the scratching behavior (Fig. 4a, b) and inflammatory factors (Fig. 4c) using the model, and the effects of topical application of anti-fungal agents. Results showed that treatment with KCZ suppressed the scratching behavior 1 h after the application of trichophythin and suppressed gene expression of histidine decarboxylase (HDC), a histamine-synthesizing enzyme. This result indicates that KCZ suppressed the degranulation of mast cells immediately after application of trichophythin, and would have inhibited scratching behavior via the downregulation of HDC. Subsequently, treatment with LNF elicited inhibition of the scratching behavior for up to 14 h and suppressed gene expression of thymic stromal lymphopoietin (TSLP). The action of LNF is to inhibit various inflammatory cytokines released from not only keratinocytes but also the infiltrating cells in the skin. Therefore, it is speculated that LNF inhibits scratching at the early phase of inflammation. Meanwhile, treatment with TBF did not show any effect on scratching behavior and inflammatory factors. These results indicate that different types of antifungal agents can regulate pruritus induced by fungal infection via different mechanisms.

**Conclusion**

We established a *Trichophyton*-induced inflammation model.
model with trichophytin, which includes components of the cell wall of *Trichophyton*. The model enabled us to study inflammation and immunological reactions, and to evaluate pruritus and the effects of anti-fungal agents on the inflammation induced by fungal components. This model may facilitate studies on the pathophysiological mechanisms of fungal infection and the development of strategies for prevention and therapies against fungal infection.
Conflict of interest

None.

This article was presented at the 62nd Annual Meeting of the Japanese Society for Medical Mycology, Tokyo, in 2018.

Fig. 2. GA inhibits trichophytin-induced ear inflammation.
(a) Statistical analysis showed that GA significantly suppressed swelling in a dose-dependent manner in trichophytin-sensitized mice challenged with antigen. Data represent the mean ± SEM of 6 animals per group. *p < 0.05, **p < 0.01 versus vehicle (trichophytin in saline).
(b) H&E staining of ear tissue sampled 48 h after trichophytin challenge. Representative histology is presented.
(c) Total RNA was extracted, and gene expression was analyzed using real-time PCR. The control was set as 1. Statistical analysis showed that GA significantly suppressed trichophytin-induced gene expression of IFN-γ, IL-6, and Mip-2 mRNA 24 h after trichophytin challenge. Data represent the mean ± SEM of 4-5 animals per group. *p < 0.05, **p < 0.01 versus vehicle.
The color version is in J-STAGE (https://doi.org/10.3314/mmj.19.005).
Fig. 3. Scratching behavior was induced by injecting trichophytin into a trichophytin-sensitized mouse.

(a) Image of itch-evoked scratching following injection of trichophytin in a trichophytin-sensitized mouse.
(b) The number of scratching behavior events was evaluated using an automatic counting device. Scratching behavior was counted until 14 h after induction of itching. Data represent the mean ± SEM of 8 animals per group. *p < 0.05, **p < 0.01 compared with the control.

Fig. 4. Anti-fungal agents and dexamethasone (DEX) suppressed trichophytin-induced scratching behavior and inflammation.

(a) The number of scratching behaviors was evaluated using an automatic counting device. Scratching behavior was counted until 1 h after induction. Data represent the mean ± SEM of 8 animals per group. **p < 0.01 compared with the control.
(b) The number of scratching behaviors was evaluated using an automatic counting device. Scratching behavior was counted until 14 h after induction of itching. Data represent the mean ± SEM of 8 animals per group. **p < 0.01 compared with the control.
(c) Ear tissues were collected 24 h after trichophytin challenge or vehicle application from trichophytin-sensitized mice, and total RNA was extracted. Gene expression was analyzed using real-time PCR. Analysis was done by comparison to the level in the vehicle-treated mice, set as 1. Data represent mean ± SEM of 6 animals per group. *p < 0.05, **p < 0.01 compared with the vehicle.
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