Optimization of a murine infection model with *Trichophyton mentagrophytes* genotype IV for studying the pathogenesis of dermatophytosis

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Introduction: Dermatophytoses are the most common cutaneous mycoses, affecting both humans and animals. The emergence of antifungal resistances highlights the need for a better understanding of dermatophyte pathogenesis and host-–pathogen interactions. Reliable animal models are therefore essential to study infection mechanisms. Using dermatophyte strains naturally associated with rodents may better reproduce infection in murine models. The aim of this study was to test a strain of *Trichophyton mentagrophytes* isolated from a rodent in a murine skin infection model, to analyze host and fungal gene expression and investigate the role of subtilisin 6 (SUB6) in virulence while mimicking natural infection conditions.

Methods: *Trichophyton mentagrophytes* TIMM 2789, originally isolated from chinchilla, was used to establish a new epicutaneous mouse infection model. Kinetic monitoring of infection was performed by establishing a global clinical score based on the intensity (0 to 4) of three clinical signs, *i.e.* erythema, scaling and crusting. Skin biopsies from infected mice were collected for histological analysis to assess fungal invasion of skin tissues, and gene expression levels of both host and fungal markers were evaluated by real-time quantitative PCR at two and five days post-infection. To assess the contribution of SUB6 to virulence, a *SUB6*-deleted (ΔSUB6) strain and a complemented strain were generated by genetic transformation and the pathogenicity of these strains was compared with that of the parental strain.

Results: Phylogenetic analysis was performed to better discriminate *T. mentagrophytes* strains isolated from animals, confirming that the TIMM 2789 strain belongs to genotype IV. Infection induced symptoms and lesions, including hair follicles invasion, typical of acute superficial dermatophytosis. Early overexpression of cytokine genes revealed the involvement of the Th1 (*IFNγ*, *IL-12β* and *TNFα*), Th2 (*IL-5*), Th17 (*IL-1β*, *IL-6*) and regulatory T cell (*IL-10*) responses by the host. Among fungal genes, a naphthalene reductase (*ARP2*), a deuterolysin (*DEUT*), a nonribosomal siderophore peptide synthase (*SIDC*) and six subtilisins (*SUB1*, *SUB3*, *SUB5*, *SUB6*, *SUB8*, and *SUB10*) were strongly upregulated during infection. Functional analysis using the ΔSUB6 mutant demonstrated that deletion of *SUB6* did not significantly reduce fungal virulence, while increased expression of *SUB5* suggested a compensatory mechanism.

Conclusions: Using a rodent-derived *T. mentagrophytes* strain in a murine infection model provides a relevant framework for studying dermatophyte infections under conditions close to natural transmission. This study highlights the activation of multiple host immune responses and identifies key fungal factors such as SIDC and SUB5 as potentially important markers during infection.

Keywords: *Trichophyton mentagrophytes*, dermatophytoses, genotyping, host cytokine, fungal virulence marker