

**TITLE:** THE EFFECT OF VIABLE OR INACTIVATED CONIDIA OF *TRICHOPHYTON RUBRUM* ON THE HUMAN GENE EXPRESSION RESPONSE DURING CO-CULTURE WITH HUMAN KERATINOCYTES

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**ABSTRACT:**

Mycoses called dermatophytosis are mainly caused by a group of filamentous fungi that invade keratinized structures causing superficial infections. The dermatophyte *Trichophyton rubrum* is the main causative agent of mycoses in skin, hair and nails. However, cases of deep dermatophytosis caused by *T. rubrum* in immunocompromised and diabetic patients as well as in individuals without immunodeficiency are also registered. Despite the importance of dermatophytosis in clinical mycology, there is still little knowledge of human genes involved in the host immune response against dermatophytes. We performed a Dual RNA-seq of human keratinocytes co-cultured with *T. rubrum* and 369 differentially expressed human genes were identified. Among these, the genes: *CSF2*, *SLC11A1*, *RNAse7*, *SERPINE 1* (involved in the tolerance and protection of human cells against the attack of pathogens) and the *FLG* gene (encodes filaggrin) involved in the composition of the epithelial barrier, presented significant expression, and can be considered important targets in the study of dermatophyte-host interaction. This work evaluated the effect of the viability of *T. rubrum* conidia on the expression of these genes during co-culture with human keratinocytes. A suspension of *T. rubrum* conidia ( $1 \times 10^7$  conidia/mL) was performed. For inactivation, conidia were exposed to a temperature of 65°C for 30 min. Viable and inactivated conidia were co-cultured with HaCat human keratinocytes ( $2 \times 10^5$  cell/mL) for 24h in RPMI medium plus 5% of fetal bovine serum at 37°C with 5% CO<sub>2</sub>. After incubation, total RNA was extracted, and the human gene expression was analyzed by RT-qPCR. In co-culture with viable conidia the relative fold change values of gene expression were: *CSF2*: 11.50; *RNAse7*: 3.33; *SERPINE1*: 3.51; *SLC11A1*: 1.69; *FLG*: 0.48). For inactivated conidia co-culture the relative fold change values were: *CSF2*: 2.21; *RNAse7*: 1.7; *SERPINE1*: 1.8; *SLC11A1*: 0.90; *FLG*: 1.88). The results showed that the viability of conidia is important for inducing the expression of genes involved in the protection of human cells against pathogens, especially for *CSF2* (cytokine GM-CSF). The *FLG* gene was induced only when it was exposed to inactivated conidia, suggesting that it causes less disturbance in epidermal barrier constituents. We conclude that genes of the innate immune system are less modulated in human keratinocytes in the presence of inactivated conidia of *T. rubrum*.

**Keywords:** dermatophytosis, epithelial barrier, fungus-host interaction, innate immunity, keratinocytes.

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