Talaromyces Marneffei Inhibits Antifungal Functions Through Affecting M2 Polarization Mediated by SOCS3-STAT6 and TLR9 Pathways in Human THP-1 Macrophages

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Abstract

Talaromyces marneffei (*T. marneffei*) is an emerging opportunistic infection in AIDS patients who lacking functional adaptive immunity. Macrophages are the predominant phagocytic cells that is resist to *T. marneffei* infection, and polarization state of macrophages is critical for the antifungal functions. However, how *T. marneffei* infection affects human macrophages polarization remains poorly understood. Here we showed that *T. marneffei* affects human THP-1 macrophages antifungal functions by inducing M2 polarization. We identified that SOCS3 is a positive regulator of M1 polarization in human THP-1 macrophages and plays an important role in limiting M2 polarization by inhibiting SATA6. Also, we found that TLR9 is required for *T.marneffei*-induced human THP-1 macrophages M2 polarization. Importantly, mechanism research found that *T. marneffei* infection directly reduce SOCS3 production via increasing SOCS3 protein tyrosine phosphorylation, while activating TLR9 pathway, thereby inducing human THP-1 macrophages M2 polarization. In conclusion, *T. marneffei* suppresses antifungal activities through human THP-1 macrophages M2 polarization, which regulating by SOCS3-STAT6 and TLR9 pathways. Our founding provides a novel mechanism for *T. marneffei*-infected AIDS patients to suppress disseminated transmission.

Methods

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Paque Plus (GE Healthcare) density centrifugation according to the manufacturer's instructions. The human monocytic cell line THP-1 was purchased, with 50 ng/ml PMA for 72 hours for THP-1 differentiation. *T. marneffei* strain L0 was separated from a HIV/TM co-infectious patient and identified by morphology and PCR analysis of ITS rDNA sequences. For phagocytosis assay, *T. marneffei* spores were stained with calcofluor white for 10 min at room temperature, and macrophages were stain with CFSE for 30 min. Then, the THP-1 differentiated macrophages were coincubated with *T. marneffei* for 24h at 37°C and 5% CO2 and the numbers of CFU were counted an 24 h of incubation at 30°C. Next, the quantitative real-time PCR, western blot and flow cytometry were conducted to detect the expression of various factors at the transcription and translation levels respectively. The immunoprecipitation was used to analyse expression level of SOCS3. Finally, the statistical significance between two groups and among multiple groups by Student's t test and one-way analysis variance (ANOVA), respectively. Data were showed as mean ± SD. The p value < 0.05 was considered significant.

Results

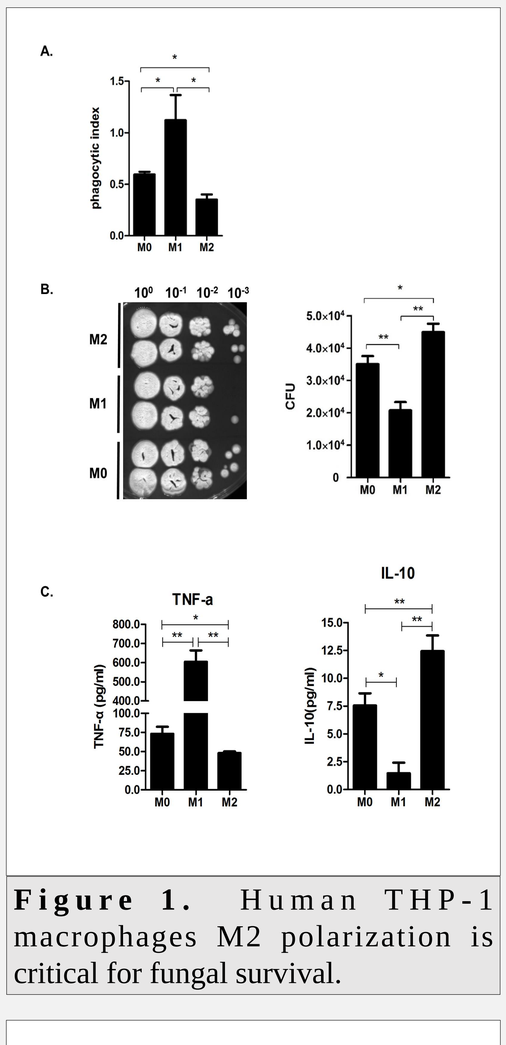

Figure 1. Human THP-1 macrophages M2 polarization is critical for fungal survival.

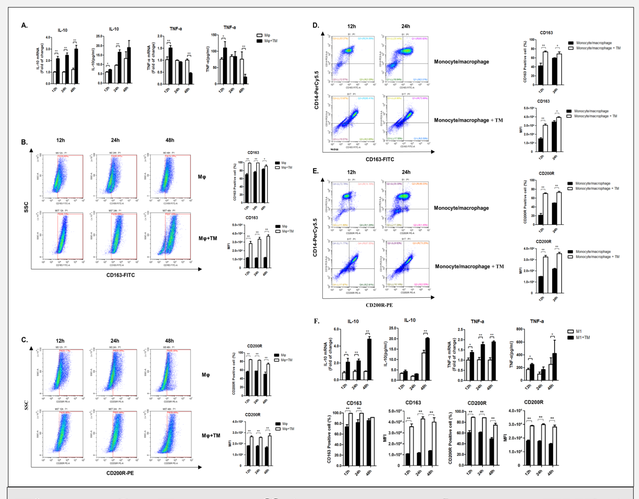

Figure 2. *T.marneffei* promotes human THP-1 macrophages M2 polarization.

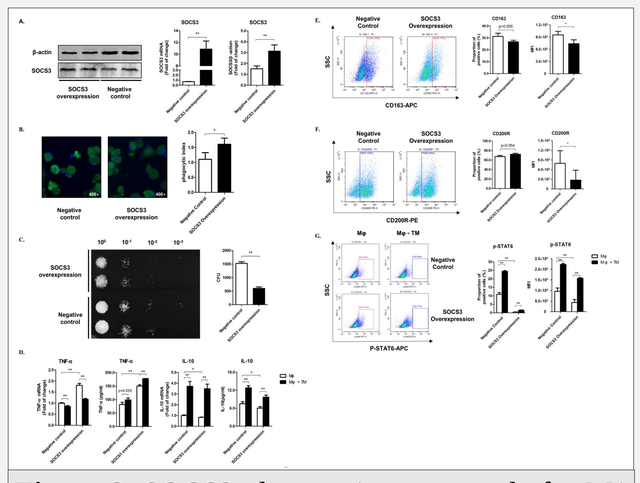

Figure 3. SOCS3 plays an important role for M1 polarization in human THP-1 macrophages.

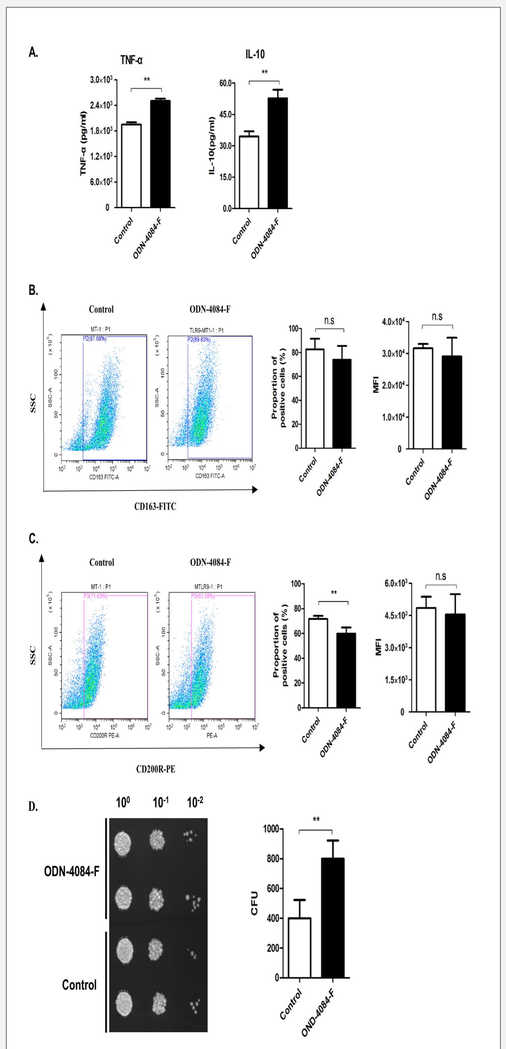

Figure 6. *T.marneffei* infection mediates human THP-1 macrophages M2 polarization by TLR9.

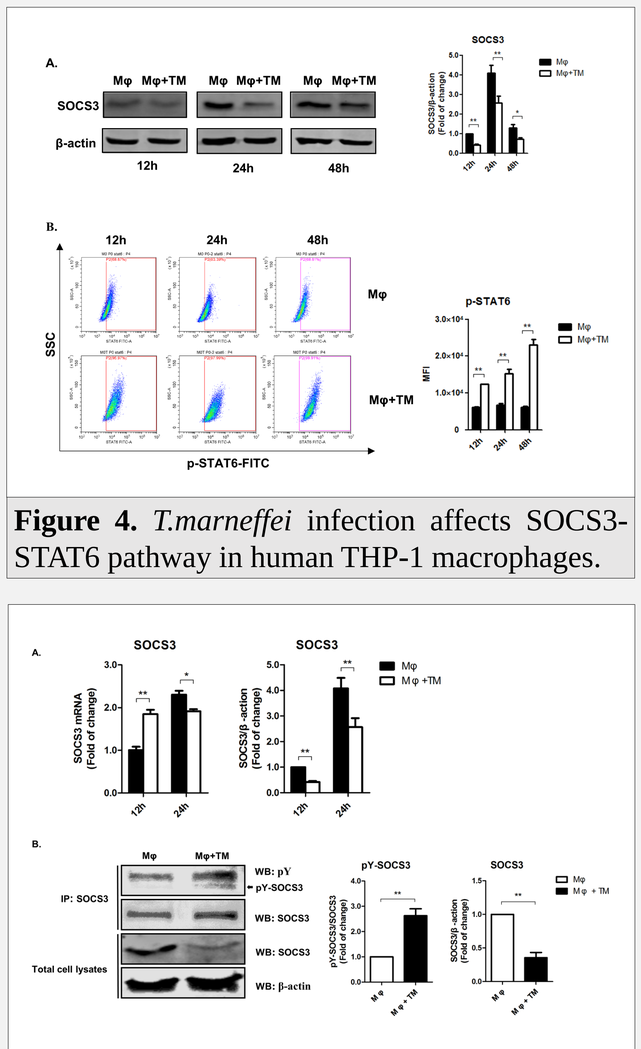

Figure 4. *T.marneffei* infection affects SOCS3-STAT6 pathway in human THP-1 macrophages.

Figure 5. *T.marneffei* infection induces SOCS3 protein tyrosine phosphorylation thereby enhancing SOCS3 protein degradation in human THP-1 macrophages.

Conclusions

We propose that *T. marneffei* infection achieves immune evasion by modulating the SOCS3-STAT6 and TLR9 pathways to induce M2 polarization in human THP-1 macrophages. Our results reveal a mechanism by which T. marneffei evades the immune response, which may provide a therapeutic target for *T. marneffei*-infected AIDS patients to inhibit disseminated transmission.