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Silencing SOCS1 in dendritic cells promote survival of mice with systemic *Candida albicans* infection via inducing Th1-cell differentiation

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ABSTRACT

Enhancing the immunity conferred by dendritic cells (DCs) to fungal infection represents a promising strategy in the number of immunocompromised individuals. In a previous study, we demonstrated that suppressor of cytokine signaling 1 (SOCS1) silencing can promote the maturation of DCs and induce an immune response against *Candida albicans (C. albicans) in vitro*. Herein, the effectiveness of SOCS1 suppression administered by SOCS1-siRNA-treated DCs is further evaluated in systemic candidiasis mouse model. The SOCS1-silenced DCs increase mouse survival and significantly decrease fungal colonization in the kidneys. We confirm that the serum IFN-γ levels in SOCS1-siRNA-treated mice are higher than in all other infected groups at the early stages of infection, which correlates with a higher differentiation of IFN-γ^+CD4$^+$ T cells (Th1) in the spleen. Meanwhile, the differentiation of IL-4-producing CD4$^+$ T (Th2) or IL-17-producing CD4$^+$ T cells (Th17 cells) remain unaffected under the same treatment, suggesting that SOCS1-silenced DCs significantly affect the IFN-γ-producing CD4$^+$ T cells (Th1). However, at the late stages of infection when the differentiation of Th1, Th2 and Th17 cells decreases in SOCS1-silenced-DCs-treated mice, all the serum cytokines (IFN-γ, IL-4 and IL-17) are also reduced. In summary, treatment of mice with SOCS1-silenced DCs can protect mice from systemic infection during the early stages and thereby increase overall survival. We conclude that the increase in Th1 response in early stages avoids the cascade inflammatory response in later stages that is known to place such a large fungal load on the kidneys and cause subsequent death.

1. Introduction

Candida albicans (*C. albicans*) is a commensal organism resided on mucosal and skin surfaces; however, it can cause various infections ranging from oral, mucotaneous infection to lethal disseminated candidiasis. The latter often occur in immunocompromised individuals [1]. As systemic *C. albicans* infection is still the leading cause of nosocomial bloodstream infection in the United States [2], it usually represents an unmet medical condition, particularly in those patients staying in the extensive care unit [3]. Antifungal therapy has been relying on both azoles and echinocandins for a few decades, however, the emergence of resistant strains to either one of two classes is definitely to limit our choices against fungal infection [4]. Because *C. albicans* vaccine is currently unavailable, strategies for enhancing host immune responses are holding a great promise[5].

The most pivotal cells in the induction of immune responses are dendritic cells (DCs) [6]. DCs are the most potential antigen-presenting cells in immune system, whose maturation and functional state determine the outcome of immune response [7]. Mature DCs prime naïve CD4$^+$ T cells into Th1, Th2, and Th17 in response to pathogens; however, immature DCs can induce anergy of naïve CD4$^+$ T cells and suppress immune response [8]. Some studies have demonstrated that enhancement of maturation of DCs promotes the viral clearance via IFN-γ production [9]. It has been well known that Th1 cells provide protection against fungal infection through IFN-γ production that requires an optimal activation of phagocytes and the generation of a protective antibody [10]. We thus speculate that the maturation of DCs in response to *C. albicans* may be an attractive way to control fungal infection.

Suppressor of cytokine signaling 1 (SOCS1) is a negative feedback

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regulator that plays a key role in the differentiation, activation, and maturation of DCs [11,12]. A number of chronic viral infections have been associated with SOCS1 upregulation [13]. DCs that highly express SOCS1 in turn modulate themselves and induce immune anergy or immune tolerance in DCs; on the other hand, silencing the SOCS1 gene in DCs with siRNA benefits DC maturity and increases antitumor immunity [14]. Therefore, the function of DCs-mediated immune response would be manipulated by regulating the expression of SOCS1 in DCs. As we observed in our previous study, suppression of the SOCS1 gene in DCs enhanced the maturation of DCs and the differentiation of Th1 cells *in vitro* in the presence of fungal cells [15].

Today, whether SOCS1-silenced DCs play an antifungal role i*n vivo* remains largely unknown. The aim of the present study is to assess the effect of silencing SOCS1 in DCs on survival of mice with systemic *C. albicans* infection. Our data show that SOCS1-silenced DCs prolonged the survival of mice with systemic *C. albicans* infection, together with a quick fungal clearance in the kidney. Furthermore, we found that siSOCS1 treatment induces differentiation of Th1 cells in response to *C. albicans* at the early stage of the infection and decreases the inflammatory cytokines at the later stage of the infection. Our findings demonstrate that silencing SOCS1 in DCs may be a promising strategy for the treatment of fungal infection.

2. Materials and methods

2.1. Mice

Female Balb/c mice and male C57 mice, 8 weeks old were housed and bred in the Institute of Dermatology, Chinese Academy of Medical Sciences (CAMS) in Nanjing, Jiangsu under specific-pathogen-free conditions. The mice were kept in standard laboratory cages at a temperature of 22 ± 4 °C and maintained on a 12:12 h light/dark cycle and supplied with standard rodent chow and water available *ad libitum*. All animal care and experimental procedures were approved by the Animal Study Committee of the Institute of Dermatology, CAMS; all methods were performed in accordance with the relevant guidelines and regulations.

2.2. Purification of bone marrow-derived DCs and small interfering RNA transfection

The purification of bone marrow-derived DCs (BMDCs) was performed as previously described [16]. Briefly, the femurs and tibiae of C57 mice were flushed and the red blood cells were lysed. Bone marrow cells were then cultured in 10-cm diameter dishes with complete culture medium containing murine GM-CSF (Peprotech, USA) for 6 days to obtain immature DCs, which then purified to > 99% by CD11c-positive immunomagnetic selection with anti-CD11c conjugated microbeads (Peprotech, USA). Purified DCs were then cultured for 6 days, and subsequently transfected with siSOCS1.

Transfection of DCs with small interfering RNA (siRNA) was carried out as our previous study [15]. The sense strand sequences of siRNA target sites designed to silence the murine SOCS1 gene were as follow: siSOCS1 #2 (5′-ACACTCACTTCCGCACCTT-3′), and control siRNA (5′-CAGCCTTCCTTCTTGGGTAT -3′). Recombinant lentivirus expression vector GV248 was used as the vector for SOCS1 siRNA. Transient transfection of siRNA was carried out with the lipofectamine 2000 regent (Invitrogen) according to the manufacturer's instructions. After 48 h of additional incubation, cells were used for experiments. The degree of gene silencing and protein was assayed by west blotting.

2.3. C. albicans strain preparation and mouse model of systemic candidiasis

C. albicans strain SC5314 was prepared as previously described [17]. Briefly, *C. albicans* (0.5 ml) was cultivated overnight in 50 ml Sabouraud dextrose agar (SDA) (BD-Difco, Franklin Lakes, NJ) on a shaker at 25 °C. After the incubation, 0.5 ml of the *C. albicans* culture was transferred into 50 ml of SAB and incubated at room temperature (25 °C) for another 4 h *C. albicans* was then washed 3 times with sterile 1 × Phosphate Buffered Saline (PBS). The yeast cells were then pelleted and re-suspended in 1 × PBS and counted using 0.5% methylene blue. The number of *C. albicans* cells were adjusted with 1 × PBS to the proper concentrations to be injected intravenously (i.v.) into each mouse (female Balb/c mice); and different dosages of *C. albicans* cells (5×10^5, 1×10^6 and 2×10^6 CFU per mouse) were injected intravenously per mouse. A total of 2×10^6 siSOCS1-DCs or control DCs were intraperitoneal injected 1 day prior to *C. albicans* infection; Infection alone or blank (1 × PBS) injection were left as positive and naïve control group. Female mice were inoculated via the lateral tail vein and monitored for 28 days.

Uninfected and infected (with *C. albicans* cells 5×10^5 per mouse) mice were euthanized at days 1, 4 or 7 post-infection and the following analyses were performed: tissue fungal burdens; FACS analyses on spleen cells; histopathological analyses of the kidney; quantification of cytokines and chemokines in the blood serum by Luminex array.

2.4. Analysis of numbers of colony forming unit (CFU)

After the mice were sacrificed, organs (kidneys, brains, spleen, and livers) were removed aseptically, weighed, and homogenized in PBS using a tissue homogenizer. Serial dilutions of homogenates were plated on Sabouraud dextrose agar (SDA) and incubated at 37 °C for 24 h. After incubation, colonies were counted, and results were expressed as $\log_{10}$(CFU/ml) or $\log_{10}$(CFU/organ).

2.5. Histology

Mouse tissues (kidneys, brains, spleen, and livers) were fixed in 10% neutral buffered formalin, paraffin embedded, and sectioned at 4 μm thickness. Sections were counterstained with either hematoxylin and eosin (H&E) or periodic acid-Schiff (PAS).

2.6. Generation of single-cell organ suspensions for flow cytometric analysis

Cell suspension was generated as described previously [18]. Briefly, spleens at days 1, 4 and 7 post-infection were disassociated in PBS with 2% fetal calf serum and treated with 50 U/ml DNase (Roche) and 0.2 mg/ml Liberase TL enzyme (Roche) for 30 min at 37 °C. Following erythrocyte lysis by addition of ACK buffer (150 mM NH4Cl, 1 mM KHCO3, 0.1 mM EDTA), the debris was removed by straining and centrifuged, and the pellet was washed and re-suspended in PBS with 2% fetal bovine serum containing 5% FCS.

Cells suspension was performed following a 4–6 h stimulation with Cell Stimulation Cocktail plus Protein Transport Inhibitor, then were stained with anti-CD4-FITC, anti- IFN-γ-PE, anti-IL-4-PE-Cy7 and anti-IL-17-PE, anti- CD4-PE. Th1 was defined as CD4 + /IFN- γ +, Th2 as CD4 + /IL-4 +, and Th17 as CD4 + /IL-17 +. The total numbers of spleen-infiltrating live leukocytes were quantified as the number of CD3 cells in the live cell gate.

2.7. Serum cytokine assay

The blood samples were collected by orbital puncture at day 1, 4 and 7 post-infection at the dosage (*C. albicans*; 2.5×10^5 CFU/mouse) and serum was stored at −70 °C prior to analysis. The serum levels of IL-12p70, IFN-γ, IL-4, IL-5, IL-6, IL-23, IL-1 β and IL-17 were measured using a Luminex 200 analyzer (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

2.8. Statistical analysis

All data were analyzed using GraphPad Prism 6.0. Survival and