Endogenous Interleukin-12 regulates macrophage phagocytosis of Sporothrix schenckii

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Context: *Sporothrix shenckii* is a widespread dimorphic fungus that causes sporotrichosis, an acute and chronic infection of the skin and subcutaneous tissues. It displays a range of clinical forms from fixed cutaneous to systemic infection[1]. Systemic sporotrichosis occurs mainly in immunodeficient patients and can be potentially fatal[2]. In the host defense against *S. schencki*, macrophages play an important role[3-5] through both phagocytosis and oxidative processes[3-5].

IL-12 is an immuno-regulatory cytokine mainly produced by phagocytes and dendritic cells in response to different pathogens[6]. Functions of IL-12 include induction of interferon-γ (IFN-γ) production by T and natural killer cells, and polarization of $CD4^+$ T cells toward high-level IFN-γ-producing T helper 1 cells. IFN-γ in turn activates macrophages which enhance the clearance of the invading organisms[6]. Moreover, IL-12 can directly stimulate mouse peritoneal macrophages (PMΦ) to produce IFN-γ[7].

Endogenous IL-12 has been shown to be important for resistance to most bacteria, intracellular protozoa and fungal pathogens[8]. In fungal pathogens, neutralization of endogenous IL-12 increased the severity of experimental infection with *Histoplasma capsulatum*[9] and *Coccidiodes immitis*[10]. Previous studies, however, have not investigated the role of endogenous IL-12 in *S. schenckii* infection. Therefore, in this study we analyzed whether neutralizing antibodies against IL-12 exerts an effect on the phagocytic activity of PMΦ in gerbils infected with *S. schenckii*.

Methods: A *S. schenckii* strain was isolated from a patient with lymphocutaneous sporotrichosis at the Department of Dermatology (Hospital Juan I. Menchaca, Guadalajara Jalisco, Mexico). Yeast cells were obtained by culture from a brain-heart infusion[4] and subsequently used to infect gerbils, supplied by the breeding facilities (Centro de Investigación Biomédica de Occidente, Guadalajara Jalisco, México).

Ten three-months old male gerbils weighing 60-70 g were infected subcutaneously with $6x10^6$ *S. schenckii* yeast cells (SsY) in the left hind footpad. Neutralizing antibody against IL-12 (I 7642 SIGMA), were diluted in phosphate buffer solution (PBS). Five infected gerbils were intraperitoneally (i.p.) injected with 250 ng of anti-IL-12 at the same time as infection and two days after infection. Another five infected gerbils were injected with PBS alone. Additionally, five healthy control gerbils received PBS alone. Seven days post-infection, PMΦ were harvested from peritoneal cavities of gerbils[5].

Phagocytosis of SsY by freshly harvested PMΦ was assayed and the Phagocytic Index (PI) was determined[5]. Difference between groups was evaluated by Student's t-test, and a p-value < 0.05 was considered significant.

Results: PMΦ from *anti-IL-12-treated-infected* gerbils displayed a 55% of decrease in number of engulfed SsY compared with PMΦ from untreated-infected gerbils ($p< 0.0001$*) and a 70% of decrease compared to the healthy control gerbils ($p< 0.0001$); **Figure 1**.

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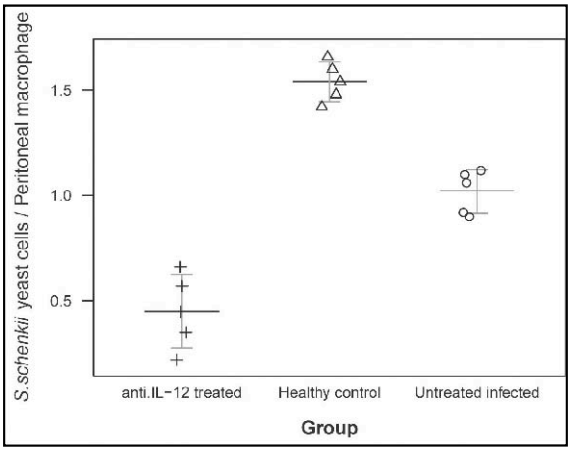

Figure 1. Phagocytic Index (PI). Values are mean +SD of experiments performed in triplicate (200 PMΦ for each experiment)

Interpretation: The results show that neutralization of endogenous IL-12 decreased macrophage phagocytosis of SsY, indicative of an impairment of host resistance to this fungus. This is in accordance with studies in experimental histoplasmosis [9] and coccidiodomycosis [10] in mice, in which neutralization of endogenous IL-12 increased the severity of infection [9, 10]. Data from this study suggest that endogenous IL-12 can exert an immunoregulatory role on phagocytic activity of PMΦ to eliminate *S. schenckii*. However, further research is required to elucidate the underlying molecular mechanisms involved in this process.

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