## 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<sup>[2]</sup>. 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<sup>+</sup>T cells toward high-level IFN-y-producing T helper 1 cells. IFN-y in turn activates macrophages which enhance the clearance of the invading organisms [6]. Moreover, IL-12 can directly stimulate mouse peritoneal macrophages (PM $\Phi$ ) to produce IFN- $\gamma^{[7]}$ .

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 PM $\Phi$  in gerbils infected with S. schenckii.

Methods: A S. schenckii strain was isolated from a 1 Unidad Académica de 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  $\frac{Recibido:}{29-Marzo-2010}$ 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,  $PM\Phi$  were harvested from peritoneal cavities of gerbils [5].

Phagocytosis of SsY by freshly harvested PM $\Phi$ 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:**  $PM\Phi$  from anti-IL-12-treated-infected gerbils displayed a 55% of decrease in number of engulfed SsY compared with PM $\Phi$  from untreatedinfected gerbils (p< 0.0001\*) and a 70% of decrease compared to the healthy control gerbils (p< 0.0001); Figure 1.

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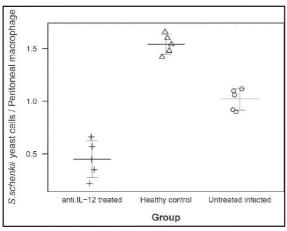


Figure 1. Phagocytic Index (PI). Values are mean +SD of experiments performed in triplicate (200 PMΦ for each experiment)

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 <sup>19</sup> and coccidiodomycosis <sup>100</sup> in mice, in which neutralization of endogenous IL-12 increased the severity of infection <sup>19, 101</sup>. Data from this study suggest that endogenous IL-12 can exert an immunoregulatory role on phagocytic activity of PM $\Phi$  to eliminate S. schenckii. However, further research is required to elucidate the underlying molecular mechanisms involved in this process.

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