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Effects of interleukin-15 on antifungal responses of human polymorphonuclear leukocytes against *Fusarium* spp. and *Scedosporium* spp.☆

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Abstract

Fusarium spp. and *Scedosporium* spp. have emerged as important fungal pathogens that are frequently resistant to antifungal compounds. We investigated the effects of human interleukin-15 (IL-15) on human polymorphonuclear leukocyte (PMNL) activity against *Fusarium solani* and *Fusarium oxysporum* as well as *Scedosporium prolificans* and *Scedosporium apiospermum*. IL-15 (100 ng/ml) significantly enhanced PMNL-induced hyphal damage of both *Fusarium* spp. and *S. prolificans* after incubation for 22 h ($P<0.01$) but not *S. apiospermum*. In addition, IL-15 enhanced PMNL oxidative respiratory burst evaluated as superoxide anion production in response to *S. prolificans* but not to the other fungi after 2 h incubation. IL-15 increased interleukin-8 (IL-8) release from PMNLs challenged with hyphae of *F. solani* and *S. prolificans* ($P \leq 0.04$). Release of tumor necrosis factor-α was not affected. The species-dependent enhancement of hyphal damage and induction of IL-8 release suggest that IL-15 plays an important role in the immunomodulation of host response to these emerging fungal pathogens.

Keywords: Filamentous fungi; Hyphal damage; Interleukin-15; Polymorphonuclear leukocytes; Oxidative burst

1. Introduction

Invasive infections due to filamentous fungi have become an important cause of morbidity and mortality in immunocompromised patients during the last two decades [1]. While the majority of these infections are caused by *Aspergillus* spp. [2], *Fusarium* spp. and *Scedosporium* spp. have emerged as less common but lethal pathogens [3–8]. Often, these emerging pathogens are more resistant to current antifungal agents, making treatment of infections due to these organisms extremely difficult. Antifungal chemotherapy alone is usually ineffective without immune recovery of phagocytic cells. Hence, understanding host immune response to these pathogens is critical for the development of more successful treatment strategies.

The innate immune response collaborating with the T helper 1 type of cytokines is the most important defense

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against invasive filamentous fungal infection and vital in the eradication of these fungal pathogens. In this regard, *Aspergillus fumigatus* is the single best-studied filamentous fungal pathogen [9,10]. While several studies have now been published on the effects of cytokines on innate immune response to *A. fumigatus*, little is known about phagocytic host defenses against *Fusarium* and *Scedosporium* spp.

Interleukin-15 (IL-15), discovered in 1994, is closely associated with the innate immune response [11,12]. IL-15 mRNA has been demonstrated in a number of cell types including macrophages [12]. In addition, it is known to act on key cells of the innate immune system including polymorphonuclear leukocytes (PMNLs) [13]. Similarly, interleukin-8 (IL-8) and tumor necrosis factor-α (TNF-α) are important cytokines involved in the recruitment and activation of phagocytes in response to infectious challenges including fungi [9,14]. Unlike IL-2, IL-15 is able to induce IL-8 release by unchallenged PMNLs and macrophages [15,16].

Therefore, we investigated the effects of IL-15 on the immune response of human PMNLs to *Fusarium* spp. and *Scedosporium* spp. Specifically, we investigated the effects of IL-15 on PMNL oxidative burst evaluated as superoxide anion (O_2^-) production, hyphal damage and production of IL-8 and TNF-α comparatively in response to the most common medically important species of *Fusarium* and *Scedosporium*: *Fusarium solani*, *Fusarium oxysporum*, *Scedosporium prolificans* and *Scedosporium apiospermum*.

2. Results

2.1. Superoxide anion production

No change in the O_2^- production occurred after IL-15 treatment when the PMNLs were unstimulated. Serum opsonization increased O_2^- production in response to *Fusarium* spp. and *Scedosporium* spp. especially at 2 h. While 2 h treatment of PMNLs with IL-15 resulted in slight enhancement of O_2^- production in response to challenge by opsonized hyphae of *S. prolificans* (Fig. 1, panel A; 1.8±0.3 to 2.0±0.3, $P=0.019$), there was no further evidence of enhanced O_2^- production by these fungi at either 2 or 22 h (Fig. 1, panel B).

2.2. Hyphal damage

Percentage hyphal damage was assessed after 2 or 22 h treatment of PMNLs with IL-15, at *E*:*T* ratios 5:1 and 10:1. IL-15 significantly enhanced PMNL-mediated damage of hyphae of both *Fusarium* spp. as well as *S. prolificans* after 22 h treatment (Fig. 2). For example, at *E*:*T* ratio 10:1, hyphal damage of *F. solani* increased from 18.2±4.5% to 28.5±5.5% ($P<0.001$, $n=8$).

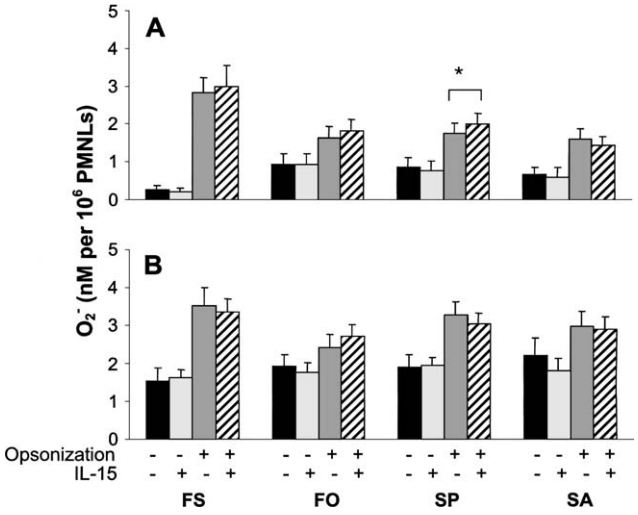

Fig. 1. Effects of incubation of human PMNLs with IL-15 for 2 h (A) or 22 h (B) on O_2^- production. FS indicates *F. solani*, FO *F. oxysporum*, SP *S. prolificans* and SA *S. apiospermum*. Cells were stimulated with non-opsonized (black and white bars) or serum-opsonized hyphae (gray and hatched bars) of *Fusarium* spp. or *Scedosporium* spp. ($n=7$). PMNLs were pre-incubated with (white and hatched bars) or without (black and gray bars) 100 ng/ml of human recombinant IL-15 for 2 h. Statistical significance ($P<0.05$) from IL-15 untreated PMNLs is indicated by * as determined by paired Student's *t*-test.

This increase was evident at both *E*:*T* ratios tested. PMNL-mediated hyphal damage was unaffected by the 2 h treatment.

2.3. Release of IL-8 and TNF-α

PMNLs incubated at 37 °C for 22 h released significantly more IL-8 in supernatants as compared to freshly prepared, unstimulated PMNLs. IL-15 treatment further enhanced the release of IL-8 from unchallenged PMNLs. There was a significant increase in release of IL-8 from IL-15 treated PMNLs challenged with *F. solani* (from 1463±501 to 4047±1334 pg/ml, $P=0.04$) and *S. prolificans* (from 1644±710 to 3193±1052 pg/ml, $P=0.02$). Additionally, there also was a consistent trend toward increased IL-8 release following stimulation with *F. oxysporum* ($P=0.13$) and *S. apiospermum* ($P=0.08$) hyphae (Fig. 3, panel A).

By comparison, IL-15 treatment did not significantly change release of TNF-α in response to any of the filamentous fungi (Fig. 3, panel B). TNF-α levels detected were in fact very low ranging from undetectable levels for untreated PMNLs to 12.2±7.9 pg/ml for *F. oxysporum*.

3. Discussion

In this study, we have demonstrated that IL-15 enhances PMNL-mediated hyphal damage of *Fusarium* spp. and *S. prolificans*, medically important fungal path-