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Lipopolysaccharide-mediated enhancement of zymosan phagocytosis by RAW 264.7 macrophages is independent of opsonins, laminarin, mannan, and complement receptor 3

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ABSTRACT

Background: Fungal and bacterial coinfections are common in surgical settings; however, little is known about the effects of polymicrobial interactions on the cellular mechanisms involved in innate immune recognition and phagocytosis.

Materials and methods: Zymosan particles, cell wall derivatives of the yeast *Saccharomyces cerevisiae*, are used to model fungal interactions with host immune cells since they display carbohydrates, including beta-glucan, that are characteristic of fungal pathogens. Using *in vitro* cell culture, RAW 264.7 macrophages were challenged with zymosan, and phagocytosis determined via light microscopy. The effects of different concentrations of lipopolysaccharide (LPS) on zymosan phagocytosis were assessed. In addition, the transfer of supernatant from LPS-treated cells to naïve cells, the effects of soluble carbohydrates laminarin, mannan, or galactomannan, and the impact of complement receptor 3 (CR3) inhibition on phagocytosis were also determined.

Results: LPS enhanced phagocytosis of zymosan in a dose-dependent manner. Transfer of supernatants from LPS-primed cells to naïve cells had no effect on phagocytosis. Laminarin inhibited zymosan phagocytosis in naïve cells but not in LPS-primed cells. Neither mannan, galactomannan, nor CR3 inhibition had a significant effect on ingestion of unopsonized zymosan in naïve or LPS-treated cells.

Conclusions: Zymosan recognition by naïve cells is inhibited by laminarin, but not mannan, galactomannan, or CR3 inhibition. LPS enhancement of phagocytosis is laminarin insensitive and not mediated by supernatant factors or zymosan engagement by the mannose or CR3 receptors. Our data suggest alternative mechanisms of zymosan recognition in the presence and absence of LPS.

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1. Introduction

Fungal infections may cause minor illnesses involving superficial structures of the mucosae or integumentary system and more severe disease with infiltration of deep-seated tissues and/or systemic invasion. Severe fungal infections are more prevalent in patients immunocompromised by human immunodeficiency virus, patients with malignancies on antineoplastic chemotherapy, and transplant patients on immunosuppressants [1,2]. Infections with fungal pathogens, particularly *Candida albicans* and *Candida glabrata*, have increased in frequency in intensive care units [3] and commonly occur concomitantly with bacterial pathogens [4]. Furthermore, increased mortality rates due to postsurgical fungal sepsis correlate with the use of multiple antibiotics, prolonged use of antibiotics, and with concomitant bacterial sepsis [5], observations which suggest the importance of understanding immune responses to fungi in the context of simultaneous bacterial infection.

Endotoxin is associated with enhanced proinflammatory mediator secretion and a cascade of reactions that often results in death [6]. In some situations, however, endotoxin exposure has been reported to augment antifungal resistance in the host. For example, when given before either inoculation with *Aspergillus fumigatus* or *Cryptococcus neoformans*, lipopolysaccharide (LPS) significantly reduced mortality and lowered fungal tissue burdens in mice [7,8]. Similar studies reported reduced *C glabrata* burdens in the tissues of mice exposed to either *Escherichia coli* or the bacterium's LPS, compared with control mice [9]. Of additional note in this latter study, was the observation of enhanced *C glabrata* phagocytosis by macrophages exposed to LPS *in vitro*. *C albicans* phagocytosis is also increased in freshly isolated human peripheral blood monocytes after stimulation with LPS [10].

Significant progress has been made in understanding the host immune response to fungal pathogens. Macrophages contribute to fungal resistance through their role as professional phagocytes by seeking out, ingesting, and killing microbes; they also secrete chemokines and cytokines, release microbicides including nitric oxide and reactive oxygen intermediates, and activate adaptive T cell immunity after fungal component antigen processing and presentation [11–13]. Macrophage recognition of pathogens and their products is achieved by host expression of germline-encoded pattern recognition receptors (PRRs); these are soluble or membrane-bound receptors which recognize microorganisms at specific conserved targets called pathogen-associated molecular patterns (PAMPs) [14]. Beta-glucans are carbohydrates found in fungi, plants, and some bacteria [15]. In fungi, they serve as significant PAMPs for several species including *C albicans* [16], *A fumigatus* [17], *Pneumocystis carinii* [18], *Coccidioides posadasii* [19], *S cerevisiae* [20], and its cell wall derivative, zymosan. Zymosan is frequently used to model fungal recognition and phagocytosis by macrophages because it is composed of large amounts of beta-glucan, in addition to small quantities of mannose and chitin [21].

We have previously reported that laminarin, a soluble low molecular weight beta-glucan, has differential effects on zymosan phagocytosis in the absence and presence of LPS [22]. Laminarin inhibited zymosan phagocytosis in the absence of LPS, consistent with beta-glucan serving as an important PAMP during macrophage recognition and phagocytosis of zymosan. The presence of LPS significantly enhanced zymosan phagocytosis, and laminarin failed to inhibit zymosan ingestion in cells primed with LPS, suggesting the presence of laminarin sensitive and insensitive mechanisms for macrophage recognition of zymosan in the absence and presence of LPS, respectively. In this study, we investigated the potential roles that any supernatant factors such as opsonins, the mannose receptor, or the complement receptor 3 (CR3) may play in contributing to the enhanced zymosan phagocytosis induced by LPS.

2. Materials and methods

2.1. Reagents

Zymosan prepared from *S cerevisiae*, laminarin from *Laminaria digitata*, mannan from *S cerevisiae*, galactomannan from the locust bean gum of *Ceratonia siliqua*, and LPS from *E coli* serotype 055:B5 were all obtained from Sigma-Aldrich (St. Louis, MO). To assess the role of the CR3 receptor in phagocytosis experiments, functional grade–purified anti-mouse CD11b antibody M1/70 (Integrin α_M or Mac-1α) and its isotype-matched rat anti-mouse IgG2b control antibody were obtained from eBioscience (San Diego, CA). For phagocytosis assays, macrophages were cultured in sterile tissue culture medium composed of Roswell Park Memorial Institute 1640 medium (Life Technologies, Burlington, ON), supplemented with 10% heat-inactivated fetal calf serum (Life Technologies), 100 U/mL penicillin (Life Technologies), 100 mg/mL streptomycin (Life Technologies), 25 mM N-2-hydroxyethylpiperazine-NV-2-ethanesulphonic acid (Life Technologies), and 10 mM L-glutamine (Life Technologies; R10). Dulbecco Modified Eagles Medium (DMEM) (Life Technologies) was used for the growth of RAW 264.7 macrophages and supplemented in the same way as the Roswell Park Memorial Institute to form D10. Sterile plasticware, noncharged, was obtained from Corning Inc (Fisher Scientific, Toronto, ON), and used for all experiments.

2.2. Macrophage cell culture

RAW 264.7 macrophages from American Tissue Type Culture Collection were obtained from Cedarlane Laboratories, Burlington, ON. Cells were maintained in D10 and passaged every 3 d when >90% confluent. In preparation for phagocytosis assays, cells were harvested and centrifuged at room temperature for 7 min at 1100 rpm, resuspended in R10, counted, and viability assessed using trypan blue dye (Sigma-Aldrich, St. Louis, MO) exclusion. Viability in all experiments was >90%.

2.3. Opsonization of zymosan

Serum opsonization of zymosan was achieved by incubating 1×10^8 zymosan particles per ml suspended in R10 with an equal volume of fetal calf serum at 37°C for 30 min with