CANDIDA ALBICANS INDUCES THE RELEASE OF INFLAMMATORY MEDIATORS FROM HUMAN PERIPHERAL BLOOD MONOCYTES

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Abstract—Candida albicans (C. albicans) is a major nosocomial pathogen. We examined arachidonic acid (AA) and cytokine production by monocytes stimulated with C. albicans. [14C]-AA labeled monocytes released 8.9 ± 2.3% of the incorporated AA following stimulation with live C. albicans (C. albicans: monocyte of 16:1) (P = 0.0002). Prior studies indicate that soluble α-mannans and β-glucans antagonize mannose and β-glucan receptors, respectively. Preincubation of monocytes with α-mannan (100 μg/ml) caused 45.8 ± 5.7% inhibition of [14C]-AA release, whereas β-glucan (100 μg/ml) yielded 43.7 ± 6.0% inhibition (P < 0.05 for each compared to control). Additionally, monocytes stimulated with C. albicans also released interleukin-1β (IL-1β), tumor necrosis factor-α (TNFα), interleukin-6 (IL-6) and interleukin-8 (IL-8). However, α-mannan or β-glucan failed to inhibit IL-1β release. These data indicate that C. albicans induces monocytes to release AA and inflammatory cytokines. Furthermore, AA, but not cytokine liberation, is partially mediated by α-mannan and β-glucan components of the fungus.

INTRODUCTION

C. albicans is among the most common nosocomial pathogens, especially in patients who are immunosuppressed, on broad-spectrum antibiotics, or who have indwelling intravascular devices [1–4]. Penetration of C. albicans into the bloodstream leads to disseminated candidiasis which has become a serious and potentially life-threatening infection, particularly in those with critical illness. The mortality of disseminated candidiasis in severely immunocompromised patients...
approaches 95% [5]. Despite the prevalent and severe nature of this infection, native host responses to this ubiquitous infectious agent are not completely understood.

A number of studies indicate that both peripheral blood monocytes and neutrophils are important components of host defense once C. albicans has entered the bloodstream [6–9]. Monocytes are known to phagocytize and degrade C. albicans and are likely more efficient than polymorphonuclear leukocytes in killing the organism [8–10]. Additional studies indicate that C. albicans can elicit release of eicosanoids and cytokines from endothelial cells as well as tissue macrophages [11, 12]. However, activation of inflammatory pathways in peripheral blood monocytes in response to C. albicans has not yet been fully studied.

Recent studies indicate that mononuclear cells interact with pathogenic fungi through at least two receptor systems, namely, the mannose and β-glucan receptors [13–16]. The cell wall of C. albicans has been shown to contain α-mannan, β-glucan, and chitin components [17–20]. Investigations reveal that mannans of C. albicans mediate adherence of yeasts to spleen and lymph node tissue [14]. In addition, C. albicans mannans have been shown to have immunodulatory effects which may explain the ability of C. albicans to persist in tissues during various disease states [17, 21–24]. Additionally, soluble β-glucans have been shown to inhibit phagocytosis and eicosanoid release from mononuclear cells stimulated with the fungal cell wall component zymosan [25–28].

This study was therefore undertaken to accomplish the following goals; 1) to quantify the release of inflammatory eicosanoids from human peripheral blood monocytes stimulated with C. albicans, 2) to determine the pattern of cytokines (IL-1β, TNFα, IL-6, and IL-8) liberated from monocytes in response to this fungus, and 3) to evaluate the potential roles of mannose and β-glucan receptors in mediating the release of inflammatory mediators from monocytes challenged with C. albicans.

**MATERIALS AND METHODS**

*Isolation of Human Peripheral Blood Monocytes.* Human peripheral blood monocytes were isolated from buffy coats as previously described [29]. Briefly, the buffy coat was diluted with HBSS (without Ca²⁺ or Mg²⁺) and subjected to gradient centrifugation over Lymphoprep (Nycomed Pharma AS, Oslo, Norway). Platelets were removed by multiple washes and centrifugation over 5% fatty acid free BSA. T-cells were removed by rosetting with sheep RBCs and the B-cells were separated by centrifugation over a Percoll gradient. The monocyte pellet was resuspended in mixed media (1:1 mixture of Medium 199 and RPMI 1640) supplemented with 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Sigma, St. Louis, Missouri) and enumerated using a hemacytometer.

*Preparation of C. Albicans.* Stock cultures of C. albicans strain (ATCC 36082, Rockville,