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Role of Mannose-Binding Lectin in the Innate Defense against *Candida albicans*: Enhancement of Complement Activation, but Lack of Opsonic Function, in Phagocytosis by Human Dendritic Cells

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Mannose-binding lectin (MBL) is a serum collectin believed to be of importance in innate immunity. We have investigated the role of MBL in the first-line defense against *Candida albicans*, an opportunistic fungal pathogen. MBL bound *C. albicans* via its lectin domain, resulting in agglutination of the organisms upon their outgrowth of hyphae. In a human *in vitro* MBL system, deposition of C4 fragments on *C. albicans* was increased when exogenous MBL was added to serum samples from MBL-deficient individuals. Similar enhancement of deposition of iC3b also was observed. MBL and enhanced opsonic C3 fragments mediated by MBL did not facilitate opsonophagocytosis of the organisms by monocyte-derived dendritic cells (DCs). However, MBL was found to inhibit the growth of *C. albicans* independently of complement activation, although, with complement activation, further inhibition was observed. We concluded that MBL plays an important role in the first-line defense against *C. albicans* without the need for opsonophagocytosis by DCs, in which a direct interaction of MBL with *C. albicans* results in agglutination and accelerated complement activation via the lectin pathway, leading to inhibition of growth.

Mannose-binding lectin (MBL) is a serum protein of hepatic origin that belongs to the collectin family [1] and plays an important role in innate immunity [2, 3]. MBL binds, through multiple lectin domains, to carbohydrate structures on microbial surfaces and is believed to mediate direct killing via complement activation [4] or via enhancing phagocytosis by acting as an opsonin [5–7]. On binding to a sugar-rich microbial surface, MBL activates the complement system via MBL-associated serine protease (MASP)–2 [8]. The ac-

tivated serine protease is then able to cleave, sequentially, C4 and C2, resulting in the generation of covalently bound C4b2a complexes, C3 convertases, which allows conversion of C3 into C3a and C3b/iC3b, the key reaction leading to initiation of the terminal pathway. Accumulating evidence has shown that MBL can modulate the host inflammatory response to infections in both *ex vivo* and *in vivo* models [6, 9–12].

In humans, a single MBL gene on chromosome 10 encodes the protein [13, 14]. MBL deficiency and low levels of serum MBL are strongly associated with the gene polymorphisms in the exon 1 [15–18] and promoter regions [19]. At present, 7 different haplotypes have been described, and 4 of these (LYPB, LYQC, HYPD, and LXPA) are associated with low levels of the protein [20]. In many populations, MBL deficiency is the most common immunodeficiency described at present, and several clinical studies have established an association between MBL deficiency and susceptibility to disease [21, 22].

Candida albicans is a component of the normal mi-

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croflora of the alimentary tract and mucocutaneous membranes of the healthy host. However, when immune defenses are compromised or the normal microflora balance is disrupted, *Candida* organisms transform into opportunistic pathogens. Indeed, dissemination of *Candida* organisms is the leading cause of invasive fungal disease in premature infants, diabetic patients, and surgical patients and of oropharyngeal disease in patients with AIDS [23–29]. *C. albicans* can switch from a unicellular yeast form into various filamentous hyphal forms, all of which can be found in infected tissues [30]. The ability of *C. albicans* to alter its cell structure from a yeast form to a hyphal form enables the fungus to adhere to host cells and penetrate tissues [31]. This dimorphic growth is thought to be an important virulence factor [32, 33]. For this to be possible, mechanisms must exist in the host defense to target such essential aspects of the pathogenicity of *C. albicans*. Recent evidence indicates that dendritic cells (DCs), which are potent professional antigen-presenting cells, can induce cell-mediated immunity on interaction with *C. albicans*, by phagocytosis, killing, and antigen presentation [34] and are also capable of sensing different forms of the fungus by discriminative production of cytokines [35, 36].

MBL has the capacity to function as an opsonin—in particular, for phagocytosis by neutrophils [6, 7]—and has been shown to strongly bind *C. albicans* [37]. The role of MBL in the host defense against *C. albicans*, however, remains unclear. In the present study, we examined the role of MBL in the innate defense against *C. albicans*, including opsonophagocytosis by DCs.

MATERIALS AND METHODS

C. albicans. *C. albicans* strain ATCC 90028 was maintained on Sabouraud dextrose agar (Difco Laboratories) at 4°C. Organisms were grown for 16 h in brain-heart infusion broth (Oxoid) at 37°C, with orbital shaking at 200 rpm before use. Under these conditions, *C. albicans* grew as a >95% pure yeast-phase population. Immediately before each experiment, organisms were harvested by centrifugation, washed 3 times in Hanks' balanced salt solution (HBSS; Gibco-BRL), and resuspended to the appropriate concentration in HBSS containing 5 mmol/L CaCl₂ and 5 mmol/L MgCl₂ (HBSS⁺⁺).

Binding of MBL to *C. albicans*. *C. albicans* yeasts (2×10^7 cells/mL) were incubated with 5 µg/mL purified MBL (a gift from Prof. M. W. Turner, Institute of Child Health, London; purified MBL is known to be noncovalently associated with MASPs in their preparation, as described elsewhere [4]), in a total volume of 50 µL of HBSS⁺⁺, for 10 min at 37°C. The yeasts were then spun for 2 min at 10,000 g, and the pellets were washed with HBSS before resuspension in fluorescein isothiocyanate (FITC)–conjugated anti-human-MBL monoclonal antibody (4 µg/mL in HBSS; HYB 131-01; AntibodyShop). Af-

ter 15 min of incubation on ice, the samples were centrifuged and washed as described above and were fixed in 1% (vol/vol) formaldehyde and 1% (wt/vol) glucose in PBS, for flow cytometry. MBL binding was measured by use of flow cytometry performed on an EPICS Elite ESP (Coulter) at a low flow rate. Data were evaluated as mean fluorescence intensity (MFI).

Negative controls were established for MBL binding by the omission of MBL. For inhibition experiments, 20 mmol/L EDTA or 2 mg/mL mannan from *Saccharomyces cerevisiae* (Sigma-Aldrich) was added to the MBL solution 5 min before the addition of MBL to the yeasts.

Influence of MBL on formation of hyphae of *C. albicans*. *C. albicans* yeasts (1×10^6 cells/mL) were incubated with or without 5 µg/mL purified MBL in 100 µL of HBSS⁺⁺ with 10% heat-inactivated fetal calf serum (FCS; Gibco-BRL), in 96-well tissue-culture plates (Corning-Costar) (100 µL/well), for up to 3 h at 37°C (5% CO₂). The use of serum-rich medium and culturing at 37°C (5% CO₂) stimulates germination of *C. albicans* yeasts. *C. albicans* was analyzed by use of light microscopy, to determine the influence of MBL on formation of hyphae. Agglutination of *C. albicans* yeast (unicellular) or hyphae (filamentous), in the presence of MBL, was also evaluated by use of flow cytometry (scatter plots) or light microscopy, respectively.

Deposition of C4 and iC3b on *C. albicans*. Serum samples were available for study from 6 MBL-deficient but generally healthy blood donors. Ethical approval was obtained for the present study from the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster. All of the blood donors were homozygous for the LYPB haplotype of the MBL gene and had undetectable levels of serum MBL (<0.05 µg/mL), which were determined as described elsewhere [38, 39]. The freshly prepared MBL-deficient serum samples from these individuals were aliquoted and stored at –70°C until use.

C. albicans yeasts (2×10^7 cells/mL) were incubated in 50 µL of HBSS⁺⁺ containing 20% MBL-deficient serum supplemented with or without 5 µg/mL purified MBL, in Eppendorf tubes for 1, 2, 4, 8, 16, or 32 min at 37°C. The reaction in each tube was stopped by adding 1 mL of ice-cold HBSS containing 1% paraformaldehyde. Suspensions were spun for 2 min at 10,000 g. The supernatants were removed, and pellets were washed in 0.5 mL of HBSS before suspension in monoclonal anti-C4d (4 µg/mL in HBSS; Quidel), which recognizes C4, C4b, and C4d. After 15 min of incubation on ice, the samples were centrifuged and washed as described above and were resuspended in FITC–anti-mouse IgG1 (4 µg/mL in HBSS; BD PharMingen). The mixtures were incubated for a further 15 min on ice. Suspensions were centrifuged and washed, and samples were then fixed in 1% (vol/vol) formaldehyde and 1% (wt/vol) glucose in PBS, for flow cytometry. Deposition of C4 was evaluated for each

MBL-deficient serum sample in duplicate by use of flow cytometry performed as described above for MBL binding.

After the mixtures were incubated, as described above, for up to 32 min, deposition of iC3b was also determined in separate tubes in which monoclonal anti-iC3b (4 $\mu\text{g}/\text{mL}$ in HBSS; Quidel) and FITC-anti-mouse IgG2b (4 $\mu\text{g}/\text{mL}$ in HBSS; BD-Pharmingen) were used instead of monoclonal anti-C4d and FITC-anti-mouse IgG1, respectively. Evaluation of deposition of iC3b was also the same as for deposition of C4d (i.e., by flow cytometry).

Preparation of human DCs. DCs were prepared as described elsewhere [40]. In brief, peripheral blood was obtained from healthy blood donors, and peripheral blood mononuclear cells (PBMCs) were isolated by sedimentation over Ficoll-Paque gradient (Pharmacia Biotech). To generate immature DCs, monocytes positively selected from PBMCs by use of anti-CD14 microbeads (Mitenyi Biotec) were cultured in 24-well tissue-culture plates (Corning-Costar) (1 mL/well) at a concentration of 1×10^6 cells/mL in RPMI 1640 medium (Gibco-BRL) containing 10% FCS (Gibco-BRL), human recombinant granulocyte-macrophage colony-stimulating factor (50 ng/mL; R&D Systems), and human recombinant interleukin-4 (10 ng/mL; R&D Systems) for 7 days. At days 6–7, >95% of the cells were CD14⁺CD11c⁺HLA-DR⁺ DCs, as determined by use of 3-color flow cytometry. DCs were studied after 6–7 days of culture.

Opsonophagocytosis of *C. albicans* by DCs. *C. albicans* yeasts were labeled with green-fluorescent dye PKH67 (Sigma), in accordance with the manufacturer's protocol. Phagocytosis was evaluated by use of an MOI of 5 organisms:1 DC. PKH67-labeled yeasts (2×10^7 cells/mL) were incubated in 125 μL of HBSS⁺⁺ containing 20% MBL-deficient serum supplemented with or without purified MBL (1 or 5 $\mu\text{g}/\text{mL}$), in Eppendorf tubes for 16 min at 37°C. The reaction was stopped by adding 1 mL of ice-cold HBSS. The suspensions were spun for 2 min at 10,000 g. The supernatants were discarded, and the pellets were resuspended in 0.25 mL of serum-free RPMI 1640 medium (10×10^6 cells/mL) and then were transferred to 24-well tissue-culture plates (Corning-Costar) (0.25 mL/well). DCs (2×10^6 cells/mL) in 0.25-mL aliquots, in serum-free RPMI 1640 medium, were added, and the mixtures, in a final volume of 0.5 mL, were incubated for 1 h at 37°C with orbital shaking at 150 rpm. A 1-mm volume of ice-cold HBSS was added to stop phagocytosis. The samples were spun, and pellets were resuspended in 200 μL of trypan blue (Gibco-BRL) (1 mg/mL in HBSS) and incubated for 10 min at 25°C, to quench the fluorescence of bound, but not engulfed, organisms, as described elsewhere [34]. The samples then were washed and fixed in 1% (vol/vol) formaldehyde and 1% (wt/vol) glucose in PBS, for flow cytometry. Phagocytosis of the PKH67-labeled *C. albicans* was evaluated by determining the percentage of DCs associated with these labeled organisms in the entire population of DCs. The

maximum percentages obtained within an experiment were assigned a value of 100%, with all other percentages within the same experiment expressed as a relative percentage of this maximum. Negative controls were established for opsonophagocytosis of *C. albicans*, by incubating organisms in HBSS⁺⁺ containing neither serum nor purified MBL in the opsonization step.

Influence of MBL on growth of *C. albicans*. Freshly grown *C. albicans* yeasts (2×10^7 cells/mL) were incubated with or without 5 $\mu\text{g}/\text{mL}$ purified MBL in HBSS⁺⁺ for 15 min at 37°C. The yeast cells were washed with HBSS, resuspended in RPMI 1640 medium at 1×10^6 cells/mL, and then transferred to 96-well tissue-culture plates (50 $\mu\text{L}/\text{well}$). To each well was added 50 μL of 2.5% MBL-deficient serum diluted in RPMI 1640 medium, 2.5% MBL-deficient serum heat inactivated for 30 min at 56°C and diluted in RPMI 1640 medium, or RPMI 1640 medium alone. The final concentration of serum used (1.25%/well) was determined to be the highest concentration giving minimal growth-inhibitory effect on *C. albicans* by our separate experiments in which yeast cells were incubated with a range of concentrations (0.625%–80%) of pooled human serum serially diluted in RPMI 1640 medium or with RPMI alone, for 6 h 37°C, and the growth of *C. albicans* was determined by use of the XTT-based cell proliferation assay (XTT-assay; Roche), as adapted from previous reports [41, 42]. The yeasts, in a final volume of 100 μL of culture medium/well, were incubated at 37°C (5% CO₂). After 3 and 6 h of incubation, the growth of *C. albicans* was determined by use of the XTT-assay. In brief, 50 μL of the XTT-labeling mixture, prepared according to the manufacturer's protocol (Roche), was added to each well. The plates were then incubated in the dark for up to 2 h at 37°C (5% CO₂). A colorimetric change in the XTT-assay was then measured in terms of the absorbance at 490 nm by use of a microtiter plate reader (Model 550; Bio-Rad).

Statistical analysis. Data are expressed as mean \pm SEM. Statistical significance was determined by paired Student's *t* tests, by use of InStat (version 3.05; GraphPad Software). *P* < .05 was considered to be significant.

RESULTS

Binding of MBL to *C. albicans*. Binding of MBL to *C. albicans* was observed at 5 $\mu\text{g}/\text{mL}$, a concentration within the range of physiological levels (e.g., <0.02–10 $\mu\text{g}/\text{mL}$ in southern Chinese persons [39]) (figure 1A). The binding was markedly reduced in the absence of Ca²⁺ (figure 1B) or in the presence of mannan (figure 1C).

Influence of MBL on formation of hyphae of *C. albicans*. Light microscopic analysis revealed that, in terms of the length of hyphae, MBL (5 $\mu\text{g}/\text{mL}$) had no influence on the formation of hyphal structures. *C. albicans* yeast in the presence or absence of MBL germinated and formed hyphae within 3 h. However, in the presence of MBL, agglutination was induced upon the

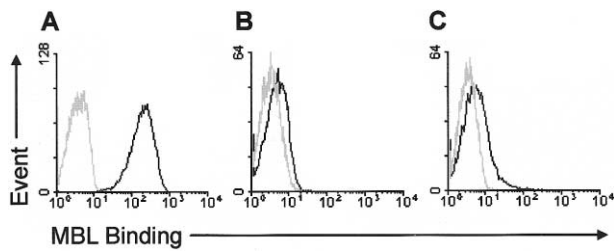


Figure 1. Ca^{2+} -dependent carbohydrate binding underlying interaction of mannose-binding lectin (MBL) with *Candida albicans*. *C. albicans* were incubated in the presence of MBL (5 $\mu\text{g}/\text{mL}$) in Hanks' balanced salt solution (HBSS) containing 50 mmol/L CaCl_2 and 50 mmol/L MgCl_2 (HBSS⁺) (A), HBSS with 20 mmol/L EDTA (B), or HBSS⁺ with 2 mg/mL mannan (C). EDTA or mannan was added to the MBL solution 5 min before addition to the yeast. After incubation with fluorescein isothiocyanate-conjugated mouse anti-human-MBL monoclonal antibody, binding of MBL to *C. albicans* was analyzed by use of flow cytometry, as shown in the representative histograms (A–C). Black lines, MBL binding; gray lines, negative controls.

outgrowth of hyphae as early as 1 h of incubation (figure 2C–2F). Such MBL-induced agglutination did not happen to *C. albicans*, which were still in the yeast phase by 20 min (figure 2A), when analyzed by use of flow cytometry (scatter plots) (figure 2B).

Deposition of C4 and iC3b on *C. albicans*. *C. albicans* yeasts were incubated with 20% serum from each of the 6 MBL-deficient individuals, to which purified MBL (5 $\mu\text{g}/\text{mL}$) was added. The time course of deposition of MBL, C4, and iC3b on the yeast cells was determined by use of flow cytometry (figure 3A). No detectable deposition of MBL was found on *C. albicans* incubated with MBL-deficient serum. Only when exogenous MBL was added to the serum did deposition of MBL become detectable (as early as 30 s), and it attained high levels of deposition over subsequent time points (up to 32 min) (figure 3B). The deposition of an early cleavage product of complement activation, C4, was detected by 1 min of incubation in serum, as shown in figure 3C. The amount of C4 appeared to be significantly enhanced by MBL at 4 min and up to 8 min ($P < .05$, paired Student's *t* test of MFI; $n = 6$). In the presence of MBL, deposition of C4 was maximal at 8 min, whereas, in the absence of MBL, deposition of C4 attained its maximal amount slowly over the time course measured. Deposition of opsonic iC3b was detected after 2 min of incubation in serum (figure 3D), and there was an enhancement of deposition in the presence of MBL, reaching significance at 16 min ($P < .05$, paired Student's *t* test of MFI; $n = 6$).

Opsonophagocytosis of *C. albicans* by DCs. PKH67-labeled *C. albicans* yeasts were incubated with 20% MBL-deficient serum supplemented with MBL (0, 1, or 5 $\mu\text{g}/\text{mL}$), for 16 min, the time point at which maximal deposition of opsonic iC3b occurred (figure 3D), followed by incubation with a suspension of

DCs for 1 h at 37°C. Opsonophagocytosis was measured by use of flow cytometry (figure 4). Opsonization of the yeasts in MBL-deficient serum supplemented with 0 or 1 $\mu\text{g}/\text{mL}$ MBL did not influence DC phagocytosis, compared with that for unopsonized yeasts. However, when opsonized in MBL-deficient serum with 5 $\mu\text{g}/\text{mL}$ MBL, the relative percentage of yeast-ingested DCs was reduced to 64% \pm 4% (vs. control, 93% \pm 3%, $P = .0018$, $n = 4$; vs. MBL-deficient serum with 0 $\mu\text{g}/\text{mL}$ MBL, 94% \pm 4%, $P < .001$, $n = 4$; paired Student's *t* test) (figure 4).

Influence of MBL on growth of *C. albicans*. Freshly grown *C. albicans* yeasts were incubated with or without 5 $\mu\text{g}/\text{mL}$ MBL and then were incubated with MBL-deficient serum or heat-inactivated MBL-deficient serum. As shown in figure 5, the pretreatment of yeasts with MBL, followed by incubation with either MBL-deficient serum or heat-inactivated MBL-deficient serum separately, resulted in profound inhibition of growth, compared with that observed when no MBL was used in the pretreatment ($P < .0001$, for both unheated and heat-inactivated serum, paired Student's *t* test of curve areas; $n = 6$).

DISCUSSION

The innate immune system is an evolutionarily ancient form of immunity and offers the main resistance to microbial pathogens during the early phase of infection [43]. At present, MBL has been defined as an effector molecule, such as complement, in mediating humoral innate immunity. The third pathway of complement activation initiated by the MBL-MASP complex, known as the lectin pathway, has been described to be highly conserved in evolution as far back as ascidians, our closest invertebrate relatives, suggesting that the lectin-based pathway played a pivotal role in innate immunity before the evolution of an adaptive immune system in jawed vertebrates [44]. Although cell-mediated innate immunity is thought to be a predominant mechanism for the first-line host defense against *C. albicans* [45–47], as a mediator in the highly conserved lectin-based pathway, MBL can strongly bind to *C. albicans* [37]. In the present study, we have sought to determine the role that MBL plays in the innate defense against *C. albicans*. The data presented here demonstrate that (1) MBL recognizes *C. albicans* via its carbohydrate-recognition domain and induces agglutination of the organisms upon their outgrowth of hyphae, (2) MBL mediates complement activation via the lectin pathway and subsequent deposition of opsonic C3 fragment on *C. albicans*, (3) MBL lacks function as an opsonin for phagocytosis of *C. albicans* by DCs, and (4) MBL inhibits the growth of *C. albicans*. These data suggest that MBL plays an important role in the first-line defense against *C. albicans* without the need for opsonophagocytosis by DCs.

We have demonstrated that MBL binds to *C. albicans* in a Ca^{2+} -dependent manner and that binding is inhibited by competing sugars, such as mannan, implying involvement of the

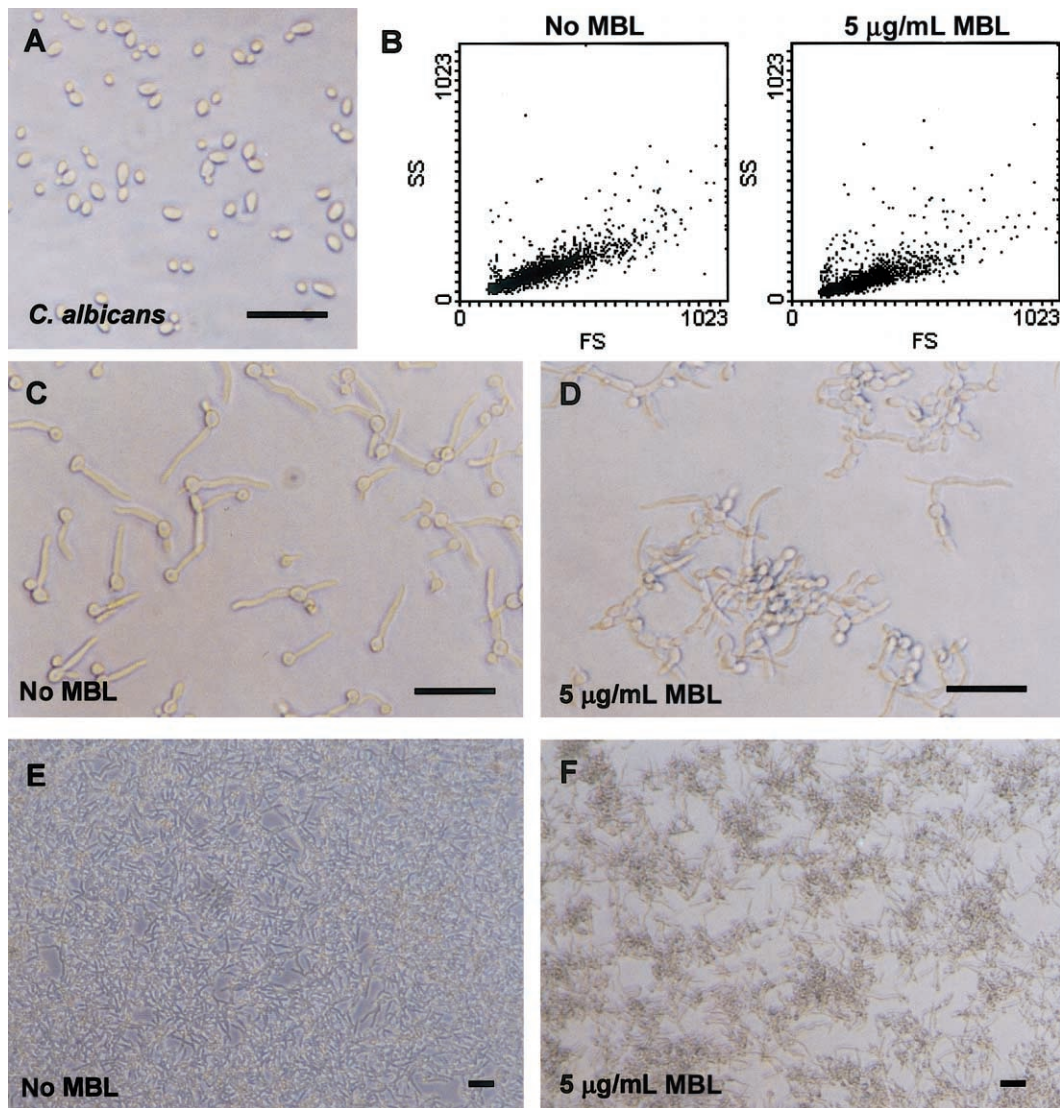


Figure 2. Agglutination induced upon the outgrowth of hyphae of *Candida albicans*, in the presence of mannose-binding lectin (MBL). *C. albicans* were incubated with MBL (0 $\mu\text{g/mL}$: *B*, left panel, *C*, and *E*; 5 $\mu\text{g/mL}$: *B*, right panel, *D*, and *F*) in Ca^{2+} -containing Hanks' balanced salt solution with 10% heat-inactivated fetal calf serum for 20 min (*A* and *B*), 1 h (*C* and *D*), and 3 h (*E* and *F*), at 37°C. Side scatter (SS) and forward scatter (FS) plots of unicellular *C. albicans* (*B*), by flow cytometry, showed no agglutination induced in the presence of MBL. Agglutination was induced upon the formation of hyphae of *C. albicans* in the presence of MBL (*D* and *F*). Bar, 20 μm , under phase-contrast light microscopy. Original magnifications: *A*, *C*, and *D*, $\times 320$; *E* and *F*, $\times 100$.

carbohydrate-recognition domain of MBL. Also, MBL does not affect the formation of hyphae of *C. albicans* but does induce agglutination upon their outgrowth of hyphae, which suggests a novel mechanism of MBL in innate immunity against *C. albicans*. Steric hindrance by MBL may effectively inhibit the spread of such a virulent form of *C. albicans* by blocking access to appropriate receptors.

C. albicans activates both the alternative and the classical pathway of complement [48], and the complement system is important in the resistance against disseminated and cutaneous candidiasis [49]. Antibodies protective against candidiasis require efficient deposition of complement on the fungal surface

[50]. In the present study, we have evaluated whether MBL mediates the lectin pathway by initiating the binding of C4 and C3 complement components to *C. albicans*. The MBL system we used to study complement function involved serum samples from MBL-deficient but generally healthy blood donors, to which exogenous MBL was added, as adapted from previous reports [4, 7]. Thus, each individual, whatever their background levels of complements fixing antibodies to the yeasts, acted as their own control. The addition of MBL to MBL-deficient serum markedly enhanced the binding of C4, reaching its maximal level at 8 min, and also enhanced the subsequent binding of iC3b to *C. albicans*, with its maximal level reached at 16

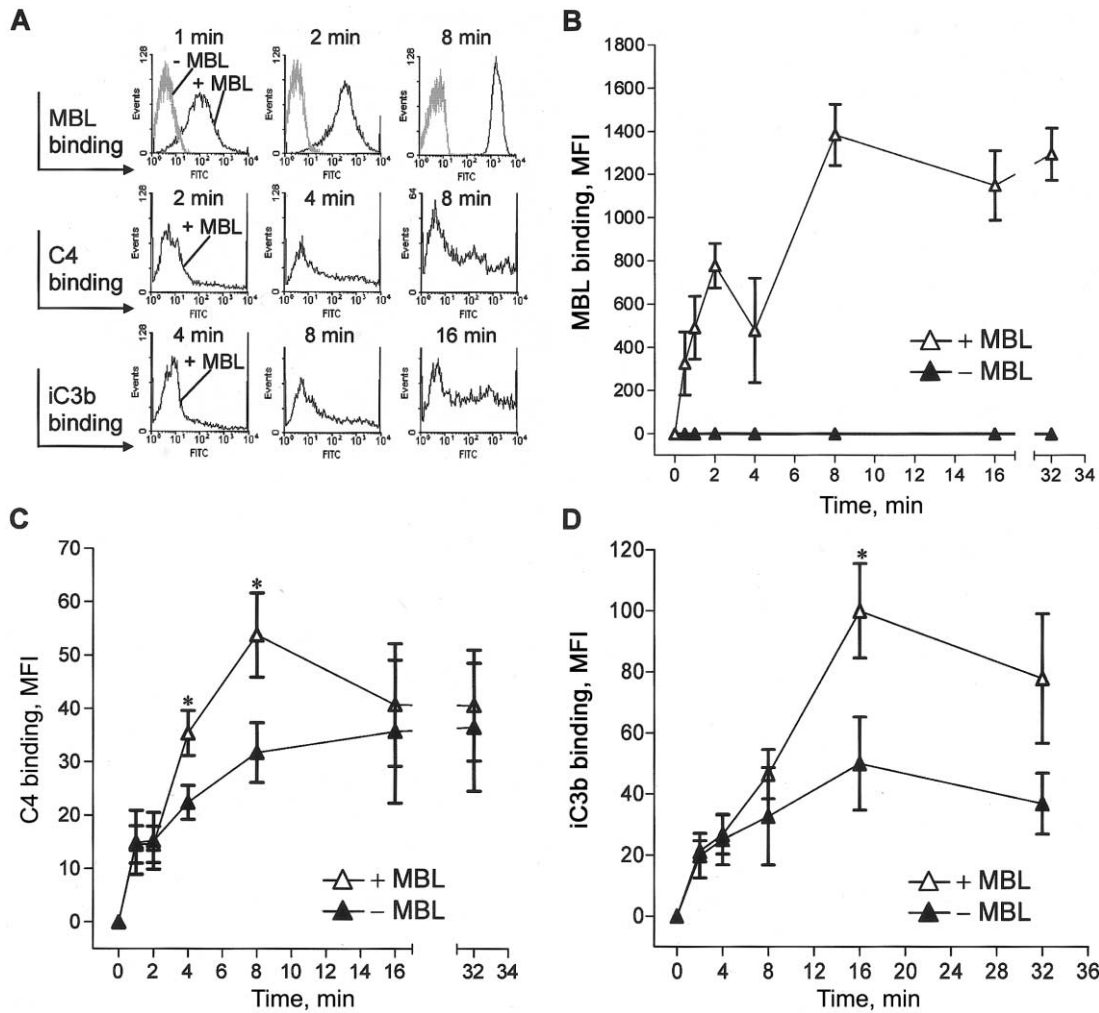


Figure 3. Time course of deposition of mannose-binding lectin (MBL), C4, and iC3b on *Candida albicans* in MBL-deficient serum supplemented with exogenous MBL. *C. albicans* were incubated with 20% MBL-deficient serum (from 6 individuals) with or without 5 $\mu\text{g}/\text{mL}$ MBL, at 37°C over the course of 32 min. The deposition was determined by use of flow cytometry, as shown in representative profiles (A). B–D, Data are the mean \pm SEM of 6 separate experiments. * $P < .05$, paired Student's *t* test of mean fluorescence intensity (MFI).

min, suggesting that MBL mediates a sequential process in complement activation via the lectin pathway independently of the individual antibodies against the yeasts.

It has become clear that various C3 fragments (e.g., C3b, iC3b, and C3d) provide alternative mechanisms for taking up microorganisms via various complement receptors on phagocytic cells [51, 52]. An early study already showed that serum with defective opsonization for phagocytosis by neutrophils has lower deposition of C3b/iC3b on *C. albicans* [53], and the presence of this opsonic defect is subsequently linked with low levels of MBL [54]. One would presume that the MBL-mediated enhancement of deposition of opsonic complement on *C. albicans*, such as that of iC3b, promotes the uptake of the fungal pathogen by phagocytic cells other than neutrophils, such as DCs. Recent studies have demonstrated an important role of DCs in mediating cellular immune response to *C. albicans* [34–

36]. In the present study, using the same MBL system as that used for the complement activation, we found that MBL did not enhance opsonophagocytosis of the yeasts by human DCs. Furthermore, opsonization by other serum factors, such as the background complements or antibodies in the serum samples from MBL-deficient individuals, appeared not to enhance phagocytosis of *C. albicans* by DCs. Newman and Holly [34] observed a similar phenomenon in their recent study in which opsonization of *C. albicans* in pooled human serum did not enhance DC phagocytosis. Our data suggest that MBL, MBL-mediated deposition of complement, and other serum factors lack opsonic function for phagocytosis of *C. albicans* by DCs. In a recent study, Turner et al. [7] demonstrated that MBL and MBL-mediated increases in opsonic C3 fragments enhance opsonophagocytosis of *Staphylococcus aureus* by neutrophils. The role of MBL as an opsonin may thus critically depend on the

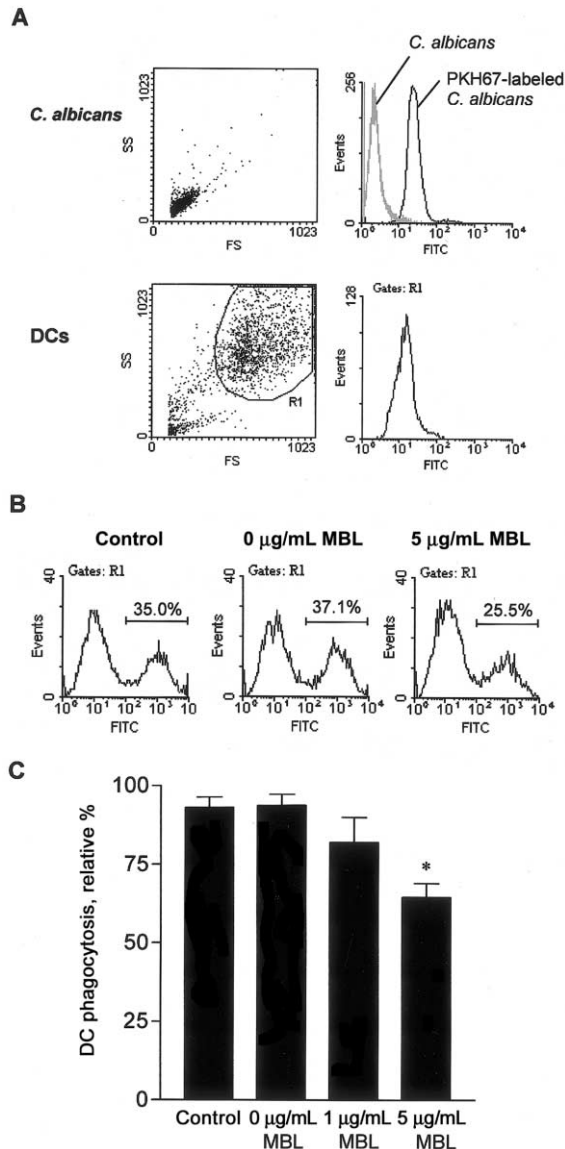


Figure 4. Opsonophagocytosis of *Candida albicans* by human monocyte-derived dendritic cells (DCs). *C. albicans* yeasts were labeled with PKH67 (A, upper panel) and preincubated in the absence (control) or presence of 20% mannose-binding lectin (MBL)-deficient serum supplemented with MBL (0, 1, or 5 $\mu\text{g/mL}$), for 16 min. Opsonized PKH67-labeled yeasts were added to a suspension of unlabeled DCs (A, lower panel) at the ratio of 5 organisms:1 DC, and the mixtures were incubated for 1 h at 37°C. Phagocytosis was then analyzed by use of flow cytometry and expressed as percentages of *C. albicans*-ingested DCs, as shown in representative profiles (B), in which fluorescence due to nonengulfed or adherent yeasts on DCs was quenched by adding trypan blue for 10 min before analysis. The maximum percentages obtained within an experiment were assigned a value of 100%, with all other percentages within the same experiment expressed as a relative percentage of this maximum (C). C, Data are the mean \pm SEM of 4 separate experiments. * $P = .0018$, vs. control (without opsonization), or $P < .001$, vs. MBL-deficient serum with 0 $\mu\text{g/mL}$ MBL, paired Student's *t* test. FITC, fluorescein isothiocyanate; FS, forward scatter; SS, side scatter.

microbial species involved and the types of phagocytes to be interacted with. DCs are capable of phagocytosing *C. albicans* in the absence of opsonins, presumably via carbohydrate-recognition receptors (also known as C-type receptors), such as mannose receptor [34, 35].

MBL, at a concentration of 5 $\mu\text{g/mL}$, was found to decrease phagocytosis of *C. albicans* by DCs, which can possibly be attributed to the large size of the agglutinated *C. albicans* complexes induced by MBL upon the outgrowth of hyphae during the incubation period of the phagocytosis assay. The decrease can also be the result of MBL interfering with the recognition of *C. albicans* by the DC C-type receptors, which mediate subsequent phagocytosis. Similar interference with the uptake of *C. albicans* was observed on macrophages in an early study using rabbit MBL [55]. Further clarification of whether MBL participates in the regulation of cell-mediated immune response is required if we are to fully understand the exact mechanisms that involve MBL in the modulation of the DC-microbe interaction.

We next considered whether the increased rate of complement activation due to MBL could result in an inhibitory effect on the growth of *C. albicans*. MBL profoundly inhibited the growth of *C. albicans* when added to MBL-deficient serum during an incubation period of up to 6 h. Interestingly, we also observed a significant inhibition of growth of *C. albicans* in the presence of MBL when the serum was heat inactivated, a situation in which complement activation would not occur. This suggests that MBL may have an intrinsic effect in inhibiting fungal growth, but the maximal inhibition we observed with unheated but MBL-supplemented serum indeed demonstrated that complement activation via the lectin pathway is of importance in inhibiting the growth of *C. albicans*. A recent study has shown that the growth of *C. dubliniensis*, a *Candida* species with phenotypical similarity to *C. albicans*, is decreased in the presence of normal serum but not in the presence of C6/C7-depleted serum [56], indicating a possible involvement of complement activation up to the terminal complement system (i.e., the generation of the membrane attack complex) in inhibiting the growth of *Candida* species. It is also possible that the availability of substrate to the organism becomes limited when *C. albicans* are agglutinated with MBL upon their outgrowth of hyphae during the time of growing. Such inhibition of growth of *C. albicans* has also been observed with another member of the collectin family, surfactant protein-D [57].

Lee et al. [58] recently demonstrated that, in mice deficient in MBL-A, resistance to disseminated candidiasis is not altered, suggesting the possibility that MBL deficiency can be compensated by functional redundancy. Two hypotheses have been proposed for the role of MBL. One of these envisages a major role of the protein during the "window of vulnerability" during early life [54]. The alternative hypothesis is that MBL is important on primary contact with an organism, by providing

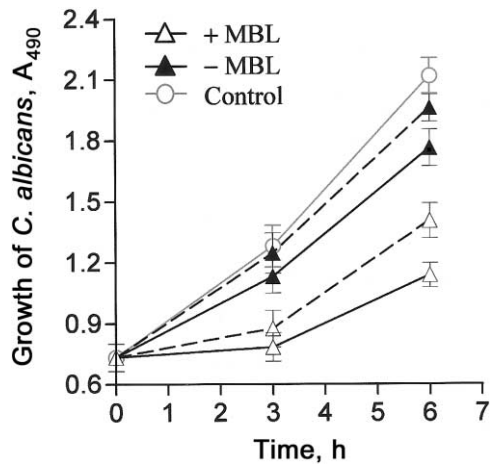


Figure 5. Influence of mannose-binding lectin (MBL) on growth of *Candida albicans*. *C. albicans* yeasts were incubated with or without 5 μ g/mL purified MBL for 15 min at 37°C. After washing, yeast cells were resuspended in RPMI with 1.25% MBL-deficient serum. After 3 h and 6 h of incubation at 37°C, the growth of *C. albicans* was determined by use of the XTT-based cell proliferation assay (see Materials and Methods). *Solid lines*, experiments using MBL-deficient serum; *dashed lines*, experiments using the same serum, heat inactivated at 56°C; and *gray line*, control experiments in which yeasts were incubated without MBL and serum. Data are plotted as mean \pm SEM ($n = 6$) of 3 separate experiments. A_{490} , absorbance at 490 nm.

humoral defense before antibody appears—so-called “ante-antibody” [59]. In the present study, we used the MBL system with exogenous MBL added to MBL-deficient serum, which controls for the biological activities due to the background levels of serum factors, such as complements and antibodies. Our observations suggest a predominant role of MBL, among other serum factors, at the early phase of defense against *C. albicans*. Although MBL deficiency is one of the most common human immunodeficiency states [22], invasive fungal infection is only seen frequently in certain individuals, such as premature infants, surgical patients, and patients with AIDS [23–29]; hence, MBL may be critical only in certain clinical situations. Previous studies have indicated the possible role of MBL in individuals with immunocompromised conditions who are susceptible to *Candida* infection [60–65]. Further research targeted at the relative importance of various mechanisms in the host defense against the fungal pathogen will help to identify the possible role of MBL replacement or adjunctive therapy in the clinical situations that render individuals susceptible to fungal infection.

In summary, the present study has documented a direct interaction between *C. albicans* and MBL, which results in accelerated complement activation via the lectin pathway, leading to inhibition of growth. We conclude that MBL has a significant influence on the host innate immunity against *C. albicans* without the need for opsonophagocytosis by DCs.

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