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Candida albicans Stimulates Arachidonic Acid Liberation from Alveolar Macrophages through α-Mannan and β-Glucan Cell Wall Components

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***Candida albicans* is an increasingly important fungal pathogen. Alveolar macrophages respond to fungal components such as zymosan by releasing arachidonic acid (AA) and AA metabolites. However, few studies have evaluated the effect of whole fungi on macrophage eicosanoid metabolism. We hypothesized that macrophages respond to *C. albicans* by releasing AA and generating AA metabolites as a consequence of interaction of mannose and β-glucan receptors with fungal cell wall components. [^{14}C]AA-labeled rabbit alveolar macrophages released AA following stimulation with either live or heat-killed *C. albicans*. High-pressure liquid chromatography analysis revealed that 55% of the AA released was metabolized via cyclooxygenase and lipoxygenase pathways. The metabolites consisted of prostaglandin E_2, prostaglandin $F_{2\alpha}$, 6-ketoprostaglandin $F_{1\alpha}$, thromboxane B_2, and leukotrienes B_4 and D_4. We further examined the roles of α-mannan and β-glucan components of *C. albicans* in mediating these alterations of eicosanoid metabolism. Prior work in our laboratory has shown that soluble α-mannan and β-glucan inhibit macrophage mannose and β-glucan receptors, respectively. Incubation of alveolar macrophages with soluble α-mannan derived from *C. albicans* (1 mg/ml) resulted in 49.8% ± 2.6% inhibition of macrophage AA release during stimulation with intact *C. albicans* ($P = 0.0001$ versus control). Macrophage AA release in response to *C. albicans* was also inhibited to a significant but lesser degree by soluble β-glucan (36.2% ± 1.3%; $P = 0.008$ versus control). These results indicate that *C. albicans* stimulates macrophage AA metabolism and that these effects are partly mediated by α-mannan and β-glucan constituents of the fungus.**

Alveolar macrophages are integral components of host defense against microorganisms which have gained access to the lower respiratory tract. Macrophage-derived eicosanoids act to initiate and modulate tissue inflammation during acute lung injury and infection (1, 5, 8, 12, 16, 17, 23, 40). Purified microbial products, such as fungus-derived zymosan, have been extensively studied and shown to be potent stimulators of eicosanoid liberation (7, 8, 18, 35). In contrast, relatively few studies have been undertaken to evaluate macrophage responses following challenge with intact fungal organisms, even though such investigations more closely approximately the interactions that occur during infection (5, 46).

Zymosan is a fungal cell wall product composed of α-mannan and β-glucan polymers (6, 14, 21, 30, 36, 43). Soluble inhibitors of macrophage mannose and β-glucan receptors have facilitated our understanding of cellular activation in response to zymosan (6, 8, 14, 21, 43). Recent studies demonstrate that β-glucan receptors on mononuclear phagocytes mediate phagocytosis and eicosanoid release (7, 20). In contrast, mannose receptors participate in phagocytosis and induction of the respiratory burst (2, 41, 43). The potential roles of these cellular receptors during interaction of alveolar macrophages with intact fungal organisms have not been fully evaluated.

Accordingly, we investigated the generation of eicosanoids from alveolar macrophages stimulated with an intact fungal organism, *Candida albicans*. *C. albicans* was studied not only because it occurs ubiquitously in the environment but in particular because it represents an increasingly prevalent cause of infection in hospitalized patients with impaired immunity (9, 10, 27, 34). In this study, we evaluated the release of arachidonic acid (AA) from alveolar macrophages stimulated with either live or heat-killed *C. albicans* cells and used high-pressure liquid chromatography (HPLC) to identify the specific eicosanoid metabolites generated. Further, the role of macrophage mannose and β-glucan receptors in mediating these alterations in macrophage eicosanoid metabolism during *C. albicans* challenge was examined by using soluble receptor antagonists.

MATERIALS AND METHODS

Isolation of resident alveolar macrophages from rabbits. Alveolar macrophages were obtained from pathogen-free New Zealand White rabbits sacrificed by intravenous injection with 50 mg of pentobarbital per kg. After the thorax was opened, the lungs were lavaged with four 50-ml aliquots of sterile, ice-cold Hanks balanced salt solution (HBSS; calcium and magnesium free) as previously described (33). The total number of cells recovered was generally in the range of 12×10^6 to 38×10^6 per animal. Cellular differentials performed on Wright-Giemsa-stained cytopreparation smears routinely indicated that lavage samples contained greater than 95% AMs. Lavage samples with any indication of bacterial or other contamination were discarded.

Incorporation of [^{14}C]AA into alveolar macrophages. Cells recovered from bronchoalveolar lavage samples were suspended in mixed media (1:1 mixture of medium 199 and RPMI

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1640 supplemented with 2 mM glutamine, 100 U of penicillin per ml, and 100 μg of streptomycin per ml) and plated onto 24-well tissue culture dishes (316,000 cells per well) as previously reported (5, 8, 33). The plates were incubated for 1 h at 37°C in a fully humidified 5% CO_2–95% air atmosphere and then gently washed with warm HBSS to remove nonadherent cells. We have previously reported that ≥95% of the macrophages are firmly adherent after this initial incubation (5). The adherent macrophages were subsequently cultured for 2 h at 37°C in mixed media containing 0.1% bovine serum albumin (BSA) and 0.1 to 0.2 μCi of [^{14}C]AA (1.9 Bq/mmol) per ml or 5 μCi of [^{3}H]AA (3.7 Bq/mmol) per ml (New England Nuclear, Boston, Mass.) as described previously (5). After incubation, the cells were washed three times with warm HBSS to remove free radiolabeled AA.

Preparation of *C. albicans*. Stock cultures of *C. albicans* ATCC 36082 were maintained at room temperature on brain heart infusion agar slants (DiMed, St. Paul, Minn.). For each experiment, suspensions of *C. albicans* were prepared in Sabouraud dextrose broth and incubated overnight at room temperature. The *C. albicans* cells were then centrifuged (2,000 × *g* for 5 min), washed with HBSS, and resuspended in mixed media. *C. albicans* blastoconidia were counted on a hemacytometer and resuspended at the indicated concentrations. In some experiments, *C. albicans* were heat killed by autoclaving at 120°C for 10 min. The heat-killed organisms were washed and similarly used to stimulate alveolar macrophages.

Preparation of other agonists and antagonists. Zymosan, derived from *Saccharomyces cerevisiae*, was prepared in phosphate-buffered saline at a concentration of 5 mg/ml by the method of Bonney (3). α-Mannan derived from *S. cerevisiae* and soluble β-glucan derived from barley (Sigma Corp., St. Louis, Mo.) were dissolved in HBSS at a concentration of 5 mg/ml. Mannans from *C. albicans* were generously provided by M. J. Herron, University of Minnesota, Minneapolis. *C. albicans* α-mannan was prepared by using cetyltrimethylammonium bromide (CTAB) by the method of Nakajima and Ballou (28). This method yields a mannan-boric acid complex which is negatively charged and forms an insoluble complex (29). In other experiments, *C. albicans* mannan was used after mild-acid treatment, accomplished by exposing *C. albicans* CTAB mannan to 10 mM HCl at 100°C for 60 min (45). This process cleaves β-1,2-linked α-mannan oligomers attached by phosphodiester linkages, resulting in monoesterified phosphates. These acid-labile β-1,2-linked oligomers are major antigenic determinants of serotype B mannan (45).

Determination of AA release from alveolar macrophages in response to *C. albicans* and zymosan. [^{14}C]AA-loaded alveolar macrophages were incubated for the times specified with *C. albicans* at the indicated concentrations in mixed media containing 0.1% BSA. In parallel wells, macrophages were incubated with zymosan (100 μg/ml), a well-established stimulant of AA liberation from macrophages. All experiments included control macrophages which were cultured in media alone. After incubation, media from the individual wells were collected and centrifuged at 1,000 × *g* for 10 min to pellet any suspended macrophages, and the supernatants were recovered. Cell monolayers were scraped and lysed in 0.5% Triton X-100 (Sigma). The counts in 300-μl aliquots from the supernatant and cell lysate samples were quantified by liquid scintillation spectroscopy. The percentage of [^{14}C]AA released from the macrophages into the media was calculated by dividing the disintegrations per minute in the supernatant by the sum of the disintegrations per minute in the supernatant and the lysate. Previous experiments in our laboratory demonstrate that macrophage viability at the time of assay is greater than 98% by Nigrosin dye exclusion (5). The average release of [^{14}C]AA from control macrophages cultured without any added stimulus was generally <5%. Unless otherwise stated, the amount of AA released from control macrophages was subtracted from each sample to correct for the spontaneous release of [^{14}C]AA.

To eliminate the possibility that either live or heat-killed *C. albicans* cells adsorb AA, we performed additional studies in which [^{14}C]AA (0.1 μCi) was incubated at 37°C for 1 h with *C. albicans* alone (0 to 50 × 10^6 of either live or heat-killed organisms) in mixed media (1 ml) containing 0.1% BSA. Subsequently, the medium was spun down, and the amount of soluble AA in the supernatant was measured. Recoverable soluble [^{14}C]AA levels were similar in incubations performed with and without live or heat-killed *C. albicans*. Thus, *C. albicans* did not significantly adsorb or incorporate [^{14}C]AA during the time course of these incubations.

HPLC analysis of AA metabolites released by macrophages stimulated with *C. albicans*. Alveolar macrophages were labeled with [^{3}H]AA as previously described (33). [^{3}H]AA-labeled alveolar macrophages were stimulated with *C. albicans* (*C. albicans*-to-macrophage ratio, 16:1) or medium alone as described above, except that the medium did not contain BSA. After 2 h of incubation, the media were extracted and analyzed by HPLC to determine the identity of AA metabolites released (22). The media from three to six wells were combined and acidified to pH 3.0 with formic acid. The acidified medium was extracted twice with ethyl acetate, dried under a nitrogen stream, redissolved in ethanol, and stored at −70°C until analysis. Prior to HPLC, the samples were diluted to 15% (vol/vol) ethanol with water and acidified to pH 3.0 to 3.5 with formic acid. The samples were then applied to C-18 octadecyl cartridges (Supelco, Bellafonte, Pa.) preconditioned with 95% ethanol followed by deionized water. The columns were sequentially washed with 15% (vol/vol) ethanol, water, and petroleum ether. The metabolites were eluted from the cartridges with methyl formate and dried under nitrogen. The residues were redissolved in 200 μl of a 1:1 mixture of 0.1% acetic acid (pH 3.7) (solvent A) and acetonitrile (solvent B). Metabolite profiles of the various samples were obtained by chromatography over a reverse-phase column (Ultrasphere octadecyl silica column; pore size, 5 μm; length, 25 cm; Beckman, Fullerton, Calif.) eluted at 1.5 ml/min with the following solvent gradient: 0 to 20 min, 33% solvent B; 20 to 22 min, linear to 50% solvent B; 22 to 30 min, 50% solvent B; 30 to 32 min, linear to 53% solvent B; 32 to 45 min, 53% solvent B; 45 to 55 min, linear to 100% solvent B. Before each sample was injected, the column was reequilibrated in 33% solvent B. During elution, UV absorbance was measured and net ^{3}H counts per minute cpm were detected by using an online radioactivity monitor (no. 171 radioisotope detector; Beckman). Metabolites were identified by comparison of their elution positions with those of unlabeled AA metabolite standards (Cayman Chemical, Ann Arbor, Mich.).

Role of mannose and β-glucan receptors in mediating AA release from alveolar macrophages stimulated with *C. albicans*. Macrophages are known to interact with fungus-derived zymosan through macrophage mannose and β-glucan receptors (6, 8, 14, 21, 43). We therefore evaluated the potential roles of macrophage mannose and β-glucan receptors in mediating AA release from macrophages during stimulation with *C. albicans*. [^{14}C]AA-labeled macrophages (316,000 per well) were incubated with soluble receptor antagonists for 30 min prior to and throughout a 90-min incubation with live *C. albicans* cells (*C. albicans*-to-macrophage ratio, 16:1). The following soluble antagonists were tested: α-mannan derived

from *S. cerevisiae*, *C. albicans* CTAB α-mannan, acid-treated *C. albicans* α-mannan, and soluble β-glucan (from barley). All antagonists were evaluated at concentrations of 100 μg/ml and 1 mg/ml. Previous studies have shown that similar concentrations of β-glucan receptor antagonists inhibit macrophage release of AA in response to zymosan (8). Again, unstimulated macrophages were cultured in mixed medium alone to determine the spontaneous release of AA. After incubation, the medium was removed and assayed for [^{14}C]AA release as described above.

Effect of soluble α-mannans and β-glucan on phagocytosis of *C. albicans* by alveolar macrophages. In addition, we sought to determine the effect of soluble macrophage receptor antagonists on impairing phagocytosis of *C. albicans* by alveolar macrophages. Phagocytic indices for macrophage uptake of *C. albicans* were determined in the presence or absence of each antagonist (CTAB α-mannan derived from *C. albicans*, α-mannan derived from *S. cerevisiae*, and soluble β-glucan; 0 to 1,000 μg/ml each) by using the method described by Bridges et al. (4). This method is based on the observation that the incorporation of radiolabeled uridine into *C. albicans* can be used as a sensitive indicator of phagocytic function since there is a linear correlation between uridine incorporation and number of yeast cells. *C. albicans* organisms within phagocytic cells do not take up uridine from the culture medium. Further, the phagocytic cells themselves do not incorporate significant amounts of uridine over short-term cultures (4). In brief, rabbit alveolar macrophages (10^6 in 0.2 ml of mixed medium containing 10% fetal calf serum) were mixed with *C. albicans* cells (2×10^6 in 0.2 ml of medium) in the presence or absence of soluble antagonist at the indicated concentrations. After 1 h of incubation at 37°C, the cultures were labeled by addition of 0.1 ml of [^{14}C]uridine (0.5 μCi/ml). The cultures were incubated for another 1 h, and the cells were washed three times with medium. Subsequently, incorporated [^{14}C]uridine was recovered by lysis with 1 N NaOH, precipitated with 20% trichloroacetic acid, and quantified by scintillation counting. The results are expressed as a phagocytic index (P.I.) calculated as the average number of *C. albicans* cells phagocytized per macrophage according to the following formula:

$$\text{P.I.} = \left[1 - \left(\frac{\text{cpm of } \textit{Candida} \text{ cells + macrophages}}{\text{cpm of } \textit{Candida} \text{ cells alone}}\right)\right] \times (\textit{Candida}/\text{macrophage ratio}) \times 100$$

The phagocytic index for macrophage uptake of *C. albicans* in the absence of any inhibitor was defined as 100% (maximal phagocytosis). Similarly, the phagocytic index of *C. albicans* maintained in culture without macrophages was 0%.

Statistical analysis. All data are expressed as the mean ± standard error of the mean from at least two to five separate experimental runs performed with different isolations of macrophages on separate occasions. Differences in measured variables between experimental and control data groups were analyzed by two-tailed Student's *t* tests. Statistical testing was performed with the Statview II statistical package (Abacus Concepts, Inc., Berkeley, Calif.) on a Macintosh IIci personal computer. Statistical differences between data were considered to be significant at $P \leq 0.05$.

RESULTS

***C. albicans* stimulates AA release from alveolar macrophages.** We first determined that alveolar macrophages release AA from membrane phospholipids when challenged with either live or heat-killed *C. albicans* cells (Fig. 1). Significant AA release was observed with 1.6 *C. albicans* blastoconidia per macrophage, which induced the release of 7.8% ± 1.0% of the incorporated [^{14}C]AA ($P = 0.0001$ compared with unstimulated control macrophages). Larger concentrations of *C. albicans* resulted in greater AA release. Rabbit alveolar macrophages stimulated with live *C. albicans* at a *C. albicans*-to-macrophage ratio of 16:1 liberated 14.6% ± 0.6% of the incorporated [^{14}C]AA ($P = 0.0001$ compared with unstimulated controls). The number of live *C. albicans* required for a half-maximal response (EC_{50}) was estimated to occur at a *C. albicans*-to-macrophage ratio of approximately 1.5:1.

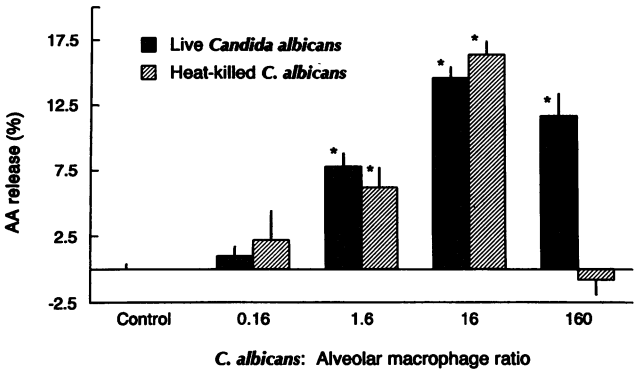

FIG. 1. Live and heat-killed *C. albicans* cells stimulate release of AA from alveolar macrophages. Normal rabbit alveolar macrophages were challenged with either live or heat-killed *C. albicans* (autoclaved at 120°C for 10 min followed by washing) at the indicated concentrations, and AA release was quantified. Both live and heat-killed *C. albicans* cells induced significant release of AA from the alveolar macrophages in a concentration-dependent fashion. Data are expressed as mean ± standard error of the mean of five experiments. *, $P < 0.05$ compared with controls.

Alveolar macrophages also released AA when stimulated with heat-killed *C. albicans*. At a *C. albicans*-to-macrophage ratio of 16:1, heat-killed organisms stimulated the release of 16.4% ± 1.0% of the incorporated [^{14}C]AA over 90 min of incubation ($P = 0.0001$ compared with control). The EC_{50} of heat-killed *C. albicans* occurred at a *C. albicans*-to-macrophage ratio of 2.1:1. In comparison, zymosan (100 μg/ml), a known potent stimulus of macrophage eicosanoid metabolism, induced the release of 14.3% ± 0.6% of [^{14}C]AA. This was similar in magnitude to the eicosanoid release induced by either live or heat-killed *C. albicans* at a concentration of 16 organisms per macrophage.

Higher concentrations of live *C. albicans* (*C. albicans*-to-macrophage ratios up to 160:1) did not result in any greater release of [^{14}C]AA from alveolar macrophages. In contrast, stimulation of macrophages with higher concentrations of heat-killed *C. albicans* resulted in an apparent suppression of AA release back to baseline levels. Interestingly, we have not previously observed any suppression of AA release even in the face of high concentrations of either yeast zymosan or particulate β-glucan (5, 8).

We next determined that alveolar macrophages challenged with *C. albicans* released AA in a time-dependent manner (Fig. 2). Significant release of AA from alveolar macrophages challenged with *C. albicans* required at least 60 min of incubation ($P < 0.05$ comparing *C. albicans*-stimulated macrophages with unstimulated controls at all time points at or exceeding 60 min). Throughout the time course, the magnitude

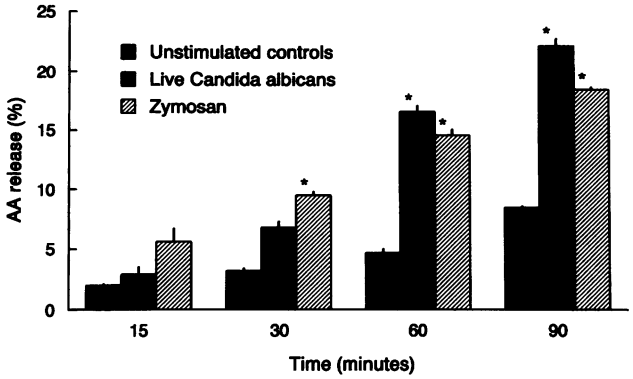

FIG. 2. *C. albicans* stimulates the release of AA from alveolar macrophages in a time-dependent fashion. Alveolar macrophages were incubated either with mixed media alone or with zymosan (100 μg/ml) or *C. albicans* (*C. albicans*-to-macrophage ratio, 16:1) for the times indicated. *C. albicans* caused alveolar macrophages to release significant amounts of AA at 60 min or more ($P < 0.05$ compared with control). Each data point represents the mean ± standard error of the mean of two separate experiments.

of AA release was similar for macrophages stimulated with either zymosan or live *C. albicans*.

AA liberated from alveolar macrophages in response to *C. albicans* is metabolized via both the cyclooxygenase and lipoxygenase pathways. We next used HPLC to evaluate the extent to which AA released from alveolar macrophages in response to *C. albicans* was subsequently metabolized via the cyclooxygenase and lipoxygenase pathways (Fig. 3). The HPLC analytical data from three experiments are integrated in Table 1. In response to *C. albicans*, 45% of the AA liberated from alveolar macrophages was released as free AA. The remaining AA (55%) was metabolized into products of the cyclooxygenase (38%) and lipoxygenase (16%) pathways. Cyclooxygenase products released from alveolar macrophages following stimulation with *C. albicans* included prostaglandins $F_{2\alpha}$, D_2, and E_2; 6-keto-prostaglandin$F_{1\alpha}$; and thromboxane B_2. The lipoxygenase products detected were leukotrienes B_4 and D_4. Our HPLC analysis did not detect any appreciable quantities of hydroxyeicosatetraenoic acids (HETEs) released from alveolar macrophages in response to *C. albicans*.

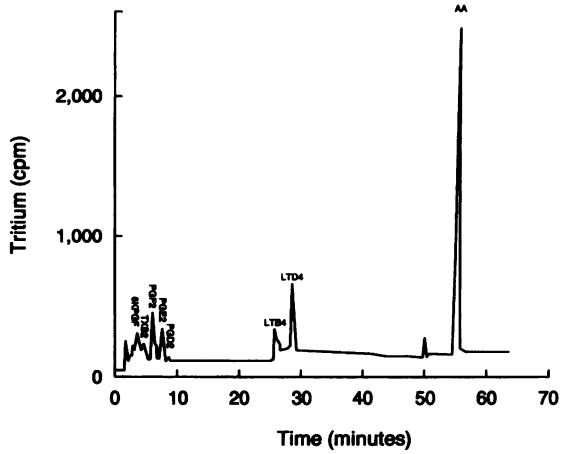

FIG. 3. Eicosanoid metabolites produced by *C. albicans* stimulation of alveolar macrophages. [^{3}H]AA-labeled rabbit alveolar macrophages were incubated with mixed medium alone or with *C. albicans* (*C. albicans*-to-macrophage ratio, 16:1) for 2 h. Supernatants were collected and analyzed by HPLC. Shown is a representative chromatographic profile identifying AA metabolites produced by alveolar macrophages stimulated with *C. albicans*. PG, prostaglandin; LT, leukotriene, TX, thromboxane.

TABLE 1. ^{3}H-eicosanoid metabolites released from alveolar macrophages stimulated with *C. albicans*[a]

AA metabolite[b]	Metabolite as % of total released from[c]:	
	Control macrophages	*C. albicans*-stimulated macrophages
6-KetoPGF$_{1\alpha}$	<1	8 ± 0.01
TXB_2	<1	5 ± 0.2
$PGF_{2\alpha}$	<1	10 ± 1.3
PGE_2	<1	10 ± 3.2
PGD_2	<1	5 ± 0.01
LTB_4	<1	6 ± 1.2
LTD_4	<1	10 ± 2.0
PTE_4	<1	<1
12-HETE/5-HETE	<1	<1
Free AA	92 ± 3	45 ± 7

[a] [^{3}H]AA-labeled rabbit alveolar macrophages were incubated with mixed medium alone (control) or with *C. albicans* (*C. albicans*-to-macrophage ratio, 16:1) for 2 h. Supernatants were collected and processed as outlined in Materials and Methods.

[b] Abbreviations: 6-ketoPGF$_{1\alpha}$, 6-keto-prostaglandin F$_{1\alpha}$; TXB_2, thromboxane B_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; PGE_2, prostaglandin E_2; PGD_2, prostaglandin D_2; LTB_4, leukotrienes B_4; LTD_4, leukotrienes D_4; LTE_4, leukotrienes E_4; HETE, hydroxyeicosatetraenoic acid.

[c] Shown are relative percentages denoting the fraction which each AA metabolite represents out of the total eicosanoids released. Stimulation of alveolar macrophages with *C. albicans* resulted in augmented generation of cyclooxygenase and lipoxygenase products. Each value represents the mean ± SEM of three experiments. It should be emphasized that the absolute magnitude of eicosanoids recovered from unstimulated control macrophages was substantially lower than the total eicosanoids released from alveolar macrophages following stimulation with *C. albicans*.

Roles of macrophage mannose and β-glucan receptors in mediating AA release following stimulation with *C. albicans*. Alveolar macrophages are known to interact with pathogenic fungi through at least two receptor types, the mannose and β-glucan receptors (11, 16). To determine the potential roles of these receptors in mediating AA release from macrophages during *Candida* infection, studies were undertaken to inhibit receptor activity with soluble receptor antagonists during macrophage challenge with live *C. albicans*.

Prior investigations have shown that mannose receptors can be inhibited by incubation with soluble α-mannans (7, 11, 16, 25, 39, 42). Therefore, soluble α-mannans were tested for their ability to inhibit AA release from macrophages challenged with *C. albicans* (Fig. 4). Notably, incubation of rabbit alveolar macrophages with *C. albicans* CTAB α-mannan prior to and throughout stimulation with *C. albicans* resulted in significant inhibition of AA release from macrophages. Alveolar macrophages incubated with organisms in the presence of soluble *C. albicans* CTAB α-mannan (100 μg/ml) exhibited a 29.7% ± 0.8% inhibition of [^{14}C]AA release ($P = 0.0001$, compared with AA release in the presence of no α-mannan). Higher concentrations of *C. albicans* CTAB α-mannan (1 mg/ml) resulted in an even larger, 49.8% ± 2.6%, inhibition of [^{14}C]AA release following *C. albicans* challenge ($P = 0.0001$). These findings suggest that α-mannan on *C. albicans* organisms interacts with macrophage receptors which mediate the release of AA.

Interestingly, acid-treated *C. albicans* CTAB α-mannan did not inhibit AA release to the same extent as did *C. albicans*

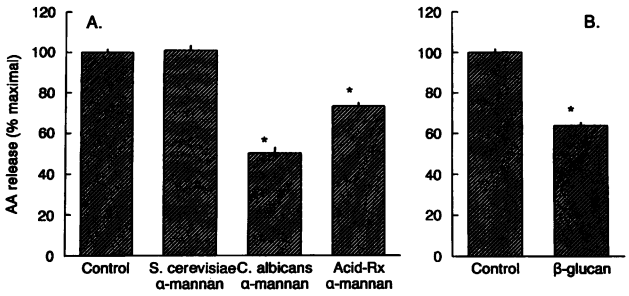

FIG. 4. Competitive antagonists of macrophage mannose and β-glucan receptors partially inhibit *C. albicans*-induced AA release from alveolar macrophages. Alveolar macrophages were incubated with either soluble α-mannan derived from *S. cerevisiae*, CTAB α-mannan from *C. albicans*, acid-treated CTAB mannan from *C. albicans*, soluble β-glucan derived from barley, or medium alone (control) throughout a 2-h incubation with *C. albicans* (*C. albicans*-to-macrophage ratio, 16:1). All data shown represent experiments in which inhibitors were used at a concentration of 1 mg/ml. (A) Effect of soluble α-mannans on *C. albicans*-stimulated AA release from macrophages. Although *S. cerevisiae* α-mannan did not alter the AA release, CTAB α-mannan derived from *C. albicans* caused a 49.8% ± 2.6% reduction in AA release ($P = 0.0001$ compared with controls). Acid treatment of *C. albicans* CTAB α-mannan partially reversed the inhibition of AA release, suggesting that β-1,2-linked oligosaccharides of *C. albicans* α-mannan may participate in macrophage AA release during *C. albicans* stimulation. (B) Effect of soluble β-glucans on *C. albicans*-stimulated AA release from macrophages. Soluble β-glucan inhibited AA release 36.2% ± 1.3% ($P = 0.008$ compared with controls). Each data point represents the mean ± standard error of the mean of three independent experiments.

CTAB α-mannan which had not been acid treated. Incubation of alveolar macrophages with *C. albicans* in the presence of acid-treated CTAB mannan caused only a 12.3% ± 1.7% inhibition of [^{14}C]AA release at 100 μg/ml ($P = 0.003$, compared with control AA release without inhibitor) and a 26.7% ± 1.6% inhibition of [^{14}C]AA release at 1 mg/ml ($P = 0.0001$). This observation further implies that fully esterified oligomeric α-mannan carbohydrates are necessary for optimal macrophage eicosanoid response.

In contrast, incubation of rabbit alveolar macrophages with α-mannan derived from *S. cerevisiae* (100 μg/ml and 1 mg/ml) failed to alter the release of [^{14}C]AA from macrophages stimulated with *C. albicans* (Fig. 4). Alveolar macrophages stimulated with *C. albicans* alone (*C. albicans*-to-macrophage ratio, 16:1) released 14.1% ± 0.8% of [^{14}C]AA compared with macrophages incubated with *C. albicans* in the presence of up to 1 mg of α-mannan per ml, which still released 14.3% ± 0.6% of the incorporated [^{14}C]AA ($P = 0.54$, not significantly different). This result may reflect differences in the α-mannan preparations from various fungal species which may differ in their ability to interact with macrophage receptors mediating changes in AA metabolism during *C. albicans* infection.

Prior studies in our laboratory demonstrate that macrophage β-glucan receptors are largely responsible for the release of AA and generation of eicosanoid metabolites induced by purified fungal zymosan (8). Treatment of alveolar macrophages with soluble β-glucan resulted in substantial inhibition of macrophage AA release following stimulation with zymosan (8). Similarly, we observed in the present study that soluble β-glucan significantly inhibited macrophage AA release following stimulation with *C. albicans*. However, the degree of inhibition was not as large as that observed with *C. albicans* α-mannan. Incubation of alveolar macrophages with *C. albicans* (*C. albicans*-to-macrophage ratio, 16:1) in the presence of soluble β-glucan caused 20.5% ± 4.8% inhibition of [^{14}C]AA release in the presence of 100 μg of soluble β-glucan per ml ($P = 0.002$ compared with AA release in the presence of no soluble β-glucan) and 36.2% ± 1.3% inhibition in the presence of 1 mg of soluble β-glucan per ml ($P = 0.008$). These data further suggest that a portion of alveolar macrophage release of AA in response to *C. albicans* may also mediated by the interaction of fungal β-glucan with macrophage receptors.

TABLE 2. Effect of soluble α-mannans and β-glucan on phagocytosis of *C. albicans* by alveolar macrophages

Soluble antagonist	Phagocytic index (%) at inhibitor concn of:		
	0 μg/ml	100 μg/ml	1,000 μg/ml
CTAB α-mannan from *C. albicans*	100.0 ± 7.3	11.0 ± 4.1[a]	10.7 ± 6.1[a]
α-Mannan from *S. cerevisiae*	100.0 ± 7.3	26.4 ± 3.8[a]	12.0 ± 10.8[a]
β-Glucan from barley	100.0 ± 7.3	<0.1 ± 1.7[a]	<0.1 ± 5.9[a]

[a] $P < 0.01$ compared with the presence of no soluble antagonist.

Of interest, additional experiments attempting to inhibit alveolar macrophage stimulation by *C. albicans* in the presence of both soluble α-mannan and β-glucan were also performed. Stimulation of macrophages with *C. albicans* in the presence of both soluble β-glucan and α-mannan did not result in significantly greater suppression of AA release compared with stimulation performed in the presence of α-mannan alone. The addition of both soluble *C. albicans* CTAB α-mannan and soluble β-glucan (1 mg/ml each) inhibited AA release by 48.2% ± 5.1% ($P = 0.56$, not significantly different from stimulations performed in the presence of α-mannan alone). Thus, the blockade of macrophage β-glucan receptors in addition to macrophage mannose receptors does not further provide additive inhibition of AA release during stimulation with *C. albicans*.

Soluble α-mannans and β-glucan inhibit phagocytosis of *C. albicans* by alveolar macrophages. Since eicosanoid release may be related to phagocytosis, we next questioned whether soluble α-mannans or β-glucan receptor antagonists inhibited phagocytosis of *C. albicans* by alveolar macrophages (Table 2). Although only CTAB α-mannan derived from *C. albicans* inhibited AA release, we observed that both α-mannan derived from *S. cerevisiae* and α-mannan derived from *C. albicans* each significantly inhibited phagocytosis ($P < 0.01$). We further observed that the magnitude of inhibition of phagocytosis was greater than the observed inhibition of AA release. For instance, whereas CATB α-mannan (1 mg/ml) inhibited 49.8% ± 2.6% of *C. albicans*-stimulated AA release, the same concentration of soluble α-mannan inhibited phagocytosis of the organism by 89.3% ± 6.1%. Further, soluble β-glucan also significantly inhibited uptake of the organism. Soluble β-glucan (100 and 1,000 μg/ml) completely inhibited *C. albicans* phagocytosis by alveolar macrophages in our assay (Table 2). These studies confirm that all the soluble macrophage receptor antagonists used in our investigation were active in antagonizing macrophage interactions with and phagocytosis of the organism.

DISCUSSION

Alveolar macrophages are primarily responsible for the initial recognition of pathogens and particulates entering the lower respiratory tract (11, 39). Although prior work has

demonstrated that zymosan, a fungal cell wall derivative, acts as a potent stimulus of macrophage eicosanoid release, few studies have evaluated macrophage eicosanoid metabolism in response to intact fungi. Our study demonstrates that *C. albicans*, a ubiquitous fungal pathogen, induces the release of arachidonic acid and its metabolites from alveolar macrophages. These data further suggest that release of AA from macrophages during challenge with *C. albicans* is mediated in part by interaction of α-mannan and β-glucan components of the fungal cell wall with cognate macrophage receptors. Prior studies by Jannace et al. have similarly demonstrated that *C. albicans* stimulates the release of arachidonate from polymorphonuclear leukocytes in culture (19).

We further observed that *C. albicans* causes the release of AA from alveolar macrophages with concentration and time characteristics similar to those observed with yeast zymosan. High concentrations of live *C. albicans* (160 organisms per macrophage) caused substantial release of AA from macrophages. In contrast, higher concentrations of heat-killed *C. albicans* resulted in minimal release of AA from macrophages. The mechanisms of this apparent suppression of AA release in the face of high concentrations of heat-killed *C. albicans* remain unclear. Recent data suggest that cellular activation may be strongly inhibited by oligosaccharides derived from *C. albicans* mannan (31, 32). It is possible that autoclaving *C. albicans* organisms results in the generation of some mannan products which inhibit AA release when present in higher concentrations. Additional investigations are required to further evaluate this possibility.

HPLC analysis of the eicosanoid metabolites released into the media from alveolar macrophages during stimulation with *C. albicans* demonstrates significant conversion of AA into cyclooxygenase and lipoxygenase products. Interestingly, the general pattern of AA metabolites released from macrophages during *C. albicans* stimulation was similar to those which we previously reported following zymosan stimulation (22). The quantities of the individual metabolites did differ to some extent. In particular, yeast zymosan resulted in somewhat greater generation of 6-keto-prostaglandin $F_{1\alpha}$ and prostaglandin E_2 from macrophages than we observed following *C. albicans* stimulation (22). In contrast, *C. albicans* induced macrophages to generate somewhat more leukotriene D_4. Our findings are highly consistent with those of Filler et al., who demonstrated significant generation of eicosanoid metabolites, mainly prostanoids, following endothelial cells stimulation by *C. albicans* (13). Only one previous study has evaluated macrophage AA metabolism in response to a fungus, *Histoplasma capsulatum* (46). In that study, Wolf et al. demonstrated significant prostanoid generation from murine peritoneal macrophages, noting a metabolite profile similar to that for zymosan-stimulated macrophages (46). Thus, it appears that fungal organisms stimulate the release from alveolar macrophages of AA which is preferentially metabolized via the cyclooxygenase system to prostanoids. In contrast, however, Kustimer has reported that *C. albicans* results in the decrease of prostaglandin E_2 and increase in leukotriene C_4 in kidney tissue during infection (24). Therefore, the pattern of eicosanoids generated in response to this organism may vary with respect to the tissue and organ affected.

Alveolar macrophages have been shown to interact with pathogenic fungi through at least two receptor systems, the macrophage mannose and β-glucan receptors (11, 16). The cell wall of *C. albicans* is composed of α-mannan, β-glucan, and chitin (15, 21, 29, 30). Our studies demonstrate that *C. albicans*-induced alterations in macrophage eicosanoid metabolism are partially, although not entirely, mediated through these receptors. Macrophage release of AA in response to *C. albicans* was inhibited 50% by α-mannan derived from *C. albicans*, strongly suggesting a role for macrophage mannose receptors in this process.

In contrast, α-mannan derived from *S. cerevisiae* failed to suppress AA release. Although prior chemical analyses demonstrate that α-mannans from *C. albicans* and *S. cerevisiae* are substantially similar, α-mannan compositions have been shown to vary among species and strains, during germ tube formation and cell growth, and even between yeast and hyphal forms of the same organism (26, 37, 38, 44). Such variations in mannan composition among strains have been related to the number of phosphate-linked side chains (45). It is possible that differences in the species carbohydrate structure, or variation in the preparations, resulted in the differing abilities of *C. albicans* and *S. cerevisiae* α-mannans to interact with macrophage receptors mediating AA release.

We further observed that acid treatment of *C. albicans* α-mannan reduces its ability to inhibit AA release from macrophages during infection with *C. albicans*. Acid hydrolysis of α-mannan has been reported to break phosphodiester linkages, causing the release of β-1,2-linked oligosaccharides from the carbohydrate (45). It is known that macrophage mannose receptors can interact with proteins containing monosaccharide residues (42). However, our findings further suggest that α-mannans which possess a full complement of β-1,2-linked oligosaccharides are necessary for optimal interaction with alveolar macrophages. The β-1,2-linked oligosaccharides of α-mannan may therefore represent additional molecular features of *C. albicans* which are necessary for optimal recognition by alveolar macrophages and release of eicosanoids.

The interaction of *C. albicans* with alveolar macrophages is undoubtedly a complex event, involving simultaneous interactions of multiple receptor-ligand pairs on the organisms and the phagocyte. Further, macrophage responses to the organism are multifaceted and involve cellular recognition, binding, phagocytosis, and stimulation of inflammatory mediator release. The extent to which these macrophage effector functions are interrelated during interaction with microorganisms is unknown. Interestingly, the data obtained from our assays of *C. albicans* phagocytosis and AA release in the presence of macrophage mannose and β-glucan receptor antagonists suggest that macrophage release of AA in response to stimulation with *C. albicans* may not always be directly coupled to uptake of the organism by the phagocyte. The interaction of macrophage receptors with specific ligands on the surface of the organisms may activate liberation of AA independently of organism phagocytosis by the macrophage.

Although studies of zymosan have yielded substantial insight into mechanisms of macrophage activation, such studies do not fully reproduce the complex interactions occurring between intact microorganisms and phagocytes. In previous studies we have investigated the role of macrophage mannose and β-glucan receptors mediating AA release from macrophages stimulated with zymosan derived from *S. cerevisiae* (8). Zymosan derived from this species is a standard fungal cell wall derivative used by a number of investigators to study cellular inflammatory responses (1, 8, 41, 43). In these earlier studies, our laboratory found that AA release from macrophages stimulated with zymosan could not be inhibited by soluble *S. cerevisiae* α-mannan. We were, however, able to inhibit AA release by using soluble β-glucans from barley (8). *S. cerevisiae* β-glucans are largely insoluble and thus are not suitable for use as inhibitors. In contrast, β-glucans derived from barley can readily be prepared in the soluble form required for a com-

petitive antagonist. Other investigators have similarly demonstrated that stimulation of monocytes with either zymosan or particulate β-glucan results in release of AA and generation of leukotrienes in a process which could be suppressed by soluble β-glucan rather than α-mannan (7). In contrast, in the present study we demonstrate that macrophage release of AA induced by intact *C. albicans* organisms can be significantly inhibited both by soluble α-mannan derived from *C. albicans* and by soluble β-glucans. Taken together, these studies suggest that the mechanisms of AA release from macrophages stimulated with the intact organism *C. albicans* differ from the response of the yeast cell wall derivative zymosan.

It should be noted, however, that eicosanoid release from macrophages during *C. albicans* stimulation is only partially mediated by these two receptor systems. Additional experiments attempting to inhibit alveolar macrophage stimulation by *C. albicans* in the presence of both soluble α-mannan and β-glucan do not result in greater than 50% suppression of AA release. It is therefore likely that additional receptor systems also participate in macrophage recognition of the fungus, in the liberation of AA, and in the generation of eicosanoid metabolites. It also remains possible that soluble β-glucan or α-mannan produces a negative signal mediated by a different mechanism that opposes the positive effect of *C. albicans* on macrophage AA release.

C. albicans is being recognized clinically as an increasing cause of nosocomial fungal infection resulting in both mucocutaneous and disseminated infections in patients with impaired cellular immunity (9, 10, 27, 34). The importance of eicosanoid metabolites as modulators of host inflammatory response during fungal infection remains to be determined. Cyclooxygenase metabolites can function as either suppressants or enhancers of host inflammatory and cellular immune responses. The products of lipoxygenase metabolism act to augment these responses. Further study is required to determine the net effect of these eicosanoids on host inflammatory responses during candidal infections in humans.

In summary, we have demonstrated that *C. albicans*, a ubiquitous fungal pathogen, elicits significant AA release from alveolar macrophages. A substantial fraction of the AA liberated is metabolized into products of the cyclooxygenase and lipoxygenase pathways, primarily the former. Alterations in macrophage eicosanoid metabolism in response to *C. albicans* are significantly suppressed by soluble *C. albicans* α-mannan containing a full complement of β-1,2-linked mannan oligomers. Macrophage release of AA is also significantly inhibited by soluble β-glucan, although to a lesser degree. Our observations suggest that mannose and β-glucan receptors participate in the alterations of arachidonic acid metabolism which occur during *C. albicans* stimulation of macrophages. Lastly, our study emphasizes the need to study the interactions of whole, living microorganisms with host cells, because they more closely approximate the interactions which occur during infection.

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