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Involvement of Mannose Receptor in Cytokine Interleukin-1β (IL-1β), IL-6, and Granulocyte-Macrophage Colony-Stimulating Factor Responses, but Not in Chemokine Macrophage Inflammatory Protein 1β (MIP-1β), MIP-2, and KC Responses, Caused by Attachment of *Candida albicans* to Macrophages

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The production of chemotactic cytokines (chemokines) and other cytokines by macrophages in response to fungal infection is thought to be critical during the course of candidiasis. However, the mechanism of cytokine synthesis by macrophages in response to fungi is not well understood. Therefore, the response of macrophages to *Candida albicans* was examined in terms of receptor-mediated chemokine and other cytokine mRNA induction. Attachment of *C. albicans* to murine thioglycollate-elicited peritoneal macrophages induced increased mRNA levels of the cytokines interleukin-1β (IL-1β), IL-6, and granulocyte-macrophage colony-stimulating factor (GM-CSF) and the chemokines macrophage inflammatory protein 1β (MIP-1β), MIP-2, and KC (a member of the platelet factor 4 neutrophil chemoattractant family), as determined by quantitative reverse transcription-PCR. However, treatment of macrophages with α-methyl-D-mannoside significantly reduced the cytokine GM-CSF response to *C. albicans* but did not affect the chemokine MIP-2 response. Antisense oligodeoxynucleotide (ODN) to mannose receptor (MR) mRNA inhibited the expression and function of MR in macrophages as determined by Western blot analysis and ^{125}I-labeled mannose-bovine serum albumin (BSA) binding, and also inhibited the elevation of cytokine IL-1β, IL-6, and GM-CSF mRNA levels induced by *C. albicans* attachment. Elevation of chemokine MIP-1β, MIP-2, and KC mRNA levels induced by *C. albicans* was not affected in macrophages whose MR expression was suppressed by antisense ODN treatment. Furthermore, IL-4 treatment of macrophages, which up-regulated MR expression as determined by Western blot analysis and fluorescein isothiocyanate-labeled mannose-BSA uptake, enhanced the level of cytokine GM-CSF mRNA induced by *C. albicans* but not the level of the chemokine MIP-2 mRNA. These results indicate that selected cytokine responses of macrophages to *C. albicans* are mediated by MR, while some chemokine responses may be mediated by other receptors.

It is widely acknowledged that cytokines, a diverse group of proteins, are important in the regulation of inflammatory responses as well as in the generation of immunity to pathogens. Recent studies have added a new superfamily, chemokine, which includes a variety of chemotactic proteins and contributes to the early inflammatory response by causing migration and activation of leukocytes, to the cytokine family (22, 29). Therefore, in this report we distinguish the terms "chemokine" and "cytokine" and designate chemotactic proteins, such as macrophage inflammatory protein 1β (MIP-1β), MIP-2, and KC, and cytokine proteins, such as interleukin-1β (IL-1β), IL-6, and granulocyte-macrophage colony-stimulating factor (GM-CSF), as chemokines and cytokines, respectively. Interaction with microbes causes secretion of a variety of cytokines and chemokines by macrophages. However, details of the induction of these cytokines and chemokines by microbes are still unclear, even though extensive studies regarding cytokine induction by microbial antigens as well as by infection have been reported (2, 12, 14, 19, 24).

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Candida albicans is a ubiquitous opportunistic yeast that causes candidiasis. The mechanism of candidiasis is not yet clear, although it has been postulated that a defect in cell-mediated immunity is critical for the disease (6). Neutrophils are extremely important in nonspecific defenses against systemic infectious disease caused by *C. albicans*, as shown by the facts that neutropenic patients are highly susceptible to development of systemic candidiasis, that the disappearance of yeast cells from tissues parallels the appearance of neutrophils, and that patients with leukocyte defects often develop candidiasis (6). In this regard, the migration of neutrophils, which may be due to chemokines, is one of the most important events during infectious disease, providing a nonspecific defense against not only *C. albicans* but also other microorganisms.

The interaction of macrophages with *C. albicans* often results in phagocytosis. Generally, phagocytosis can be divided into several fundamental steps similar to events with other microorganisms, including attachment to the macrophage surface, internalization, and endosomal processing. Cytokine and chemokine syntheses by the macrophages likely occur at each of these steps. In this regard, we showed recently that attachment of microbes to macrophages is sufficient to induce increased levels of cytokine mRNAs (34). The cell surface of a microbe, by virtue of its interaction with receptors on the macrophage surface, therefore may play a critical role in induction of cytokine synthesis during phagocytosis. However,

the precise mechanism of induction of cytokine and chemokine synthesis is not well understood.

C. albicans has a relatively simple outer structure compared with other microbes such as gram-negative bacteria. Therefore, *C. albicans* provides a useful model for macrophage-microbe interactions. Mannan, a mannose polymer, is a major component of *C. albicans* cell walls and is recognized by macrophages (17, 18). Furthermore, *C. albicans* mannan stimulates secretion of tumor necrosis factor alpha from alveolar macrophages as well, an action which is blocked by α-methyl-D-mannoside (αMM), suggesting the involvement of the mannose receptor (MR) (9). However, there is no direct evidence of MR involvement in macrophage recognition of *C. albicans* in regard to immunological responses. In the present study, we demonstrate involvement of MR in the induction of cytokine mRNA for IL-1β, IL-6, and GM-CSF but not in the induction of mRNA for the chemokines MIP-1β, MIP-2, and KC. These results indicate further that certain groups of cytokines and chemokines are regulated by different ligand-receptor interactions.

MATERIALS AND METHODS

Macrophages. Peritoneal macrophages were elicited with thioglycollate from 8- to 12-week-old female BALB/c mice (Jackson Laboratory, Bar Harbor, Maine) as described previously (38). Macrophage monolayers (10^6 or 10^5 cells per well; 24- or 96-well tissue culture plates) were cultured overnight in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS; HyClone Laboratories, Logan, Utah) and then used for experiments as described previously (35). In some experiments, macrophage monolayers were treated with 5 ng of recombinant mouse IL-4 (Genzyme, Cambridge, Mass.) per ml for 24 h at 37°C.

Yeast. *C. albicans* TIM0239 isolated from a patient with candidiasis was used in this study. The yeast was cultured on Sabouraud's dextrose agar (Difco Laboratories, Detroit, Mich.) for 24 h at 37°C and harvested into pyrogen-free saline. The number of yeast cells in a suspension was determined by hemocytometer count and adjusted to a suitable concentration with RPMI 1640 containing 10% FCS. The morphology of *C. albicans* was examined by microscopy. The yeast form was present throughout experiments.

Incubation of macrophages with *C. albicans*. Macrophage monolayers were pretreated with 1 μg of cytochalasin D (CyD) (Sigma Chemical Co., St. Louis, Mo.) per ml for 30 min and then incubated with *C. albicans* for 1 h in the presence of CyD as described previously (34). The ratio of yeasts to macrophages was 1:10.

Quantitative RT-PCR. RNA isolation from macrophages and reverse transcription (RT)-PCR were performed as described previously (35). In brief, total RNA was isolated from macrophages by the single-step method with TRI-Reagent (Molecular Research Center, Cincinnati, Ohio). RT of total RNA was performed with avian myeloblastosis virus reverse transcriptase in a commercial reaction mixture (Reverse Transcription System; Promega, Madison, Wis.). The cDNAs were subjected to differential PCR (21) with primers for β_2-microglobulin, IL-1β, IL-6, GM-CSF, MIP-1β, MIP-2, and KC. The primers for IL-1β, IL-6, and GM-CSF were purchased from Stratagene (La Jolla, Calif.). The other primers were synthesized on a DNA synthesizer (model PS250; Cruachem, Dulles, Va.) by using published sequences (7, 11, 27). The PCR was performed in a minicycler (MJ Research, Watertown, Mass.) for 30 cycles. The first cycle consisting of a 5-min denaturation at 94°C and a 5-min annealing at 60°C was followed by 30 cycles each of 1.5 min at 72°C, of 45 s at 94°C, and of 45 s at 60°C, with a final extension for 10 min at 72°C. The PCR products were quantified by high-performance liquid chromatography with a TSK DEAE-NPR column (TosoHass, Montgomeryville, Pa.). Peak absorbance (260 nm) was analyzed by computer and normalized relative to an endogenous standard (β_2-microglobulin) amplicon, which was coamplified in each sample (35). There was no significant difference in BMG mRNA levels, as shown by the amount of primer-specific products, between control and experimental groups.

Antisense ODN. The antisense phosphorothioate oligodeoxynucleotides (ODNs) of mouse MR mRNA (GenBank accession no. Z11974) were synthesized on a DNA synthesizer with 3*H*-1,2-benzodithiole-3-one, 1,1-dioxide (Glen Research, Sterling, Va.) and purified by a reverse-phase cartridge (Poly-Pak; Glen Research). The sequence of antisense ODN (MR4AS) was 5′-GAG TGG GCT TAC GTG GTT GT-3′, which targets 3′-untranslated regions (positions of nucleotide sequence, 4801 to 4820) of MR mRNA.

Antisense ODN treatment. Antisense ODN (100 nM) was incubated with 10 μg of the cationic lipid Lipofectin (Gibco, Gaithersburg, Md.) at 37°C for 5 min as described previously (4) and added to macrophage cultures supplemented with serum-free medium (QBSF 56; Sigma). The cultures were incubated for 4 h at 37°C in 5% CO_2, and the cells were overlaid with fresh medium containing antisense ODN but no Lipofectin. The cultures were incubated for 3 days with daily changes of medium containing antisense ODN. The concentration of antisense ODN used showed no significant toxicity to macrophages as determined by the tetrazolium dye reduction assay with 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (36). Untreated macrophages and macrophages treated with only Lipofectin served as controls.

MR. Determination of MR activity was done by binding and uptake assays with ^{125}I-labeled mannose-bovine serum albumin (^{125}I-mannose-BSA) or fluorescein isothiocyanate (FITC)-labeled mannose-BSA (FITC-mannose-BSA). Labeling of mannose-BSA (EY Laboratories, San Mateo, Calif.) with ^{125}I was accomplished by the chloramine-T method with an iodine labeling kit (ICN Biochemicals, Cleveland, Ohio). Macrophage monolayers (10^5 cells per well, 96-well plates) were incubated with ^{125}I-mannose-BSA (approximately 2.5×10^6 cpm) in the presence or absence of 0.5 M αMM for 1 h on ice. After incubation, macrophages were washed with phosphate-buffered saline (PBS) and lysed in 1 N NaOH, and the radioactivity of the lysates was determined in a gamma counter. FITC-mannose-BSA, FITC-glucose-BSA, and FITC-BSA were purchased from EY Laboratories. Macrophage monolayers (10^6 cells per well, 24-well plates) were incubated with 12 μg of either FITC-mannose-BSA, FITC-glucose-BSA, or FITC-BSA per 0.5 ml for 1 h at 37°C in the presence or absence of 0.1 M αMM, washed with PBS, and lysed in 0.2% sodium dodecyl sulfate (SDS), and the amount of FITC in the lysates was determined by fluorospectrometer at 482- and 520-nm wavelengths.

Western blot. The amount of MR antigen was determined by Western blotting with a polyclonal rabbit anti-mouse MR antiserum (kindly provided by P. Stahl, Washington University) as described elsewhere (26). The membrane lysates obtained from 10^7 macrophages by differential centrifugation technique were subjected to SDS–8% polyacrylamide gel electrophoresis. After electrophoresis proteins were blotted onto Hybond enhanced chemiluminescent (ECL) membranes (Amersham, Arlington Heights, Ill.) and incubated with rabbit anti-MR antiserum and anti-rabbit immunoglobulin G-peroxidase conjugate (Sigma), and reactions were detected with the ECL Western Blotting System (Amersham) as described in manufacturer's protocol.

Statistical evaluation. All experiments were repeated at least three times and evaluated by Student's *t* test for statistical significance.

RESULTS

***C. albicans* attachment to macrophages induced increased levels of cytokine and chemokine mRNAs.** Since the quantitative results of PCR are markedly affected by the experimental conditions, including even technical differences from tube to tube (5), normalization of PCR products to an endogenous standard is essential for analysis of message levels in experimental groups. Therefore, the differential PCR procedure (35), which allows comparison between an endogenous standard and a target gene in the same tube, was employed in this study. The study showed minimum constitutive message levels of the cytokines and chemokines tested with the exception of MIP-1β, which has been reported previously (32), in control macrophages. When macrophages were exposed to *C. albicans* for 1 h in the presence of CyD, which prevents uptake but permits attachment of microbes to macrophages (34), steady-state levels of mRNAs of the cytokines IL-1β, IL-6, and GM-CSF and of the chemokines MIP-1β, MIP-2, and KC were significantly increased over those in control cultures (Fig. 1). The CyD treatment alone does not have any effect on the levels of these mRNAs (34).

Effect of αMM on cytokine and chemokine induction by *C. albicans* attachment. Induction of increased levels of cytokine and chemokine mRNAs by attachment of yeast cells to macrophages may involve interactions between ligands on the yeast surface and receptors on the macrophages. Since heat-inactivated FCS was used only as a source of serum and there was no significant difference between heat-inactivated FCS-treated and untreated *C. albicans* regarding cytokine and chemokine mRNA inductions (data not shown), there were no considerable opsonins in the incubation mixture. Therefore, direct interaction between the yeast surface ligands and receptors could be detected in this assay system. Mannan is a major cell wall component of *C. albicans*. Thus, involvement of MR, which recognizes mannose preferentially (23), seems likely in the induction of cytokine and chemokine mRNA syntheses by

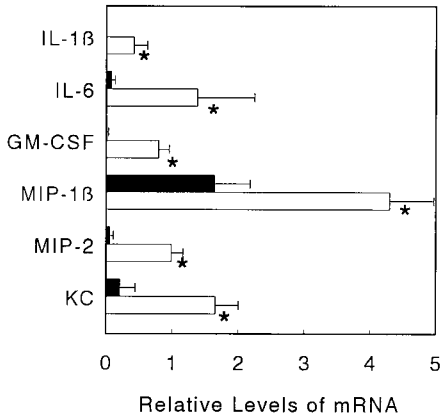

FIG. 1. Levels of cytokine and chemokine mRNAs in macrophages incubated with or without *C. albicans* as determined by quantitative RT-PCR. Macrophages were pretreated with CyD for 30 min and then incubated with or without *C. albicans* for 1 h in the presence of CyD. The ratio of yeast cells to macrophages was 1:10. Each bar represents the mean (error bar indicates ± standard error) for three experiments. ■, Control; □, with *C. albicans*; ∗, *P* of <0.05 compared with control group.

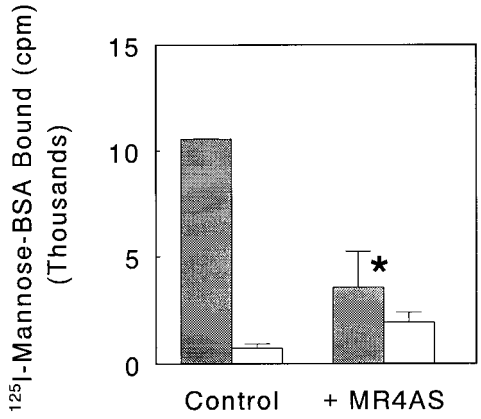

FIG. 3. Effect of antisense ODN treatment on ^{125}I-mannose-BSA bound to macrophages. Macrophages were treated with 100 nM antisense ODN (MR4AS) for 3 days as described in Materials and Methods, and then binding of ^{125}I-mannose-BSA to macrophages was determined in the presence (□) or absence (▨) of 0.5 M αMM for 1 h on ice. Each bar represents the mean (error bar indicates ± standard deviation) for three macrophage cultures. ∗, *P* of <0.05 compared with control group.

macrophages in response to attachment of *C. albicans*. αMM, a competitive inhibitor for certain lectins which interact with mannose or related carbohydrates, was used to block interactions between *C. albicans* and macrophages. GM-CSF and MIP-2 mRNAs were determined to be representative cytokine and chemokine mRNAs, respectively, because both mRNAs showed minimum constitutive expression in control macrophages and significant levels of response after yeast attachment (Fig. 1). As shown in Fig. 2, αMM treatment markedly inhibited the elevation of cytokine GM-CSF mRNA levels caused by *C. albicans* attachment to the macrophages. In contrast, elevation of chemokine MIP-2 mRNA levels was not affected by αMM treatment. αMM treatment alone had no significant effect on the levels of GM-CSF or MIP-2 mRNA in control macrophages. These results suggested that an αMM-sensitive receptor system, which may be MR, could be involved in cytokine GM-CSF induction by *C. albicans* attachment, but chemokine MIP-2 induction required an αMM-insensitive ligand-receptor interaction.

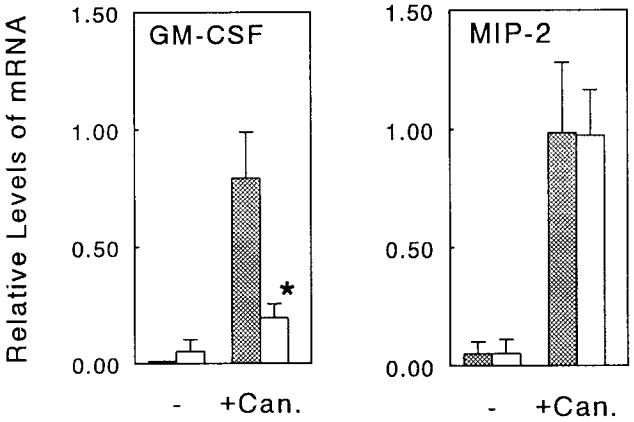

FIG. 2. Effect of αMM on cytokine GM-CSF and chemokine MIP-2 induction following attachment of *C. albicans* (Can.) to macrophages. Macrophages were pretreated with CyD for 30 min and then incubated with *C. albicans* in the presence (□) or absence (▨) of 0.1 M αMM for 1 h. ∗, *P* of <0.05 compared with the αMM-untreated group.

Down-regulation of MR by antisense ODN. In order to demonstrate the possible involvement of MR in the cytokine response of macrophages to attachment by *C. albicans*, down-regulation of MR expression was achieved with an antisense ODN. The antisense phosphorothioate ODN (MR4AS) of MR mRNA, which hybridized to the 3′-untranslated region, positions 4801 to 4820 of MR mRNA, showed the highest level of suppression of MR expression among six different antisense ODNs tested, including the initiation of the translation region and open reading frame (data not shown). The treatment of macrophages with 100 nM antisense ODN in a serum-free medium for 3 days resulted in a significant decrease of ^{125}I-mannose-BSA binding to the macrophages (Fig. 3). The binding of ^{125}I-mannose-BSA to control macrophages was inhibited approximately 93% by the addition of 0.5 M αMM, suggesting mannose-specific binding of ^{125}I-mannose-BSA. A short incubation period, such as 1 day, with antisense ODN did not significantly reduce ^{125}I-mannose-BSA binding to the macrophages (data not shown), which may be due to the biological half-life of MR (33 h) (15). The sense ODN of MR mRNA (nucleotide sequence positions 4801 to 4820) did not inhibit ^{125}I-mannose-BSA binding to the macrophages under the same experimental conditions used for the antisense ODN (data not shown).

Since a functional assay such as that for ^{125}I-mannose-BSA binding might not be MR specific, determination of specific antigen expression levels by Western blotting with an MR-specific antibody seemed useful for evaluation of MR levels in macrophages. Figure 4 shows the results of Western blotting for MR antigen in macrophage membrane lysates. As shown in the figure, an MR antigen band in the control macrophage lane is evident. The specificity of the MR band was examined by using a nonimmune rabbit serum, which resulted in no corresponding band (data not shown). The antisense ODN treatment (100 nM) for 3 days reduced the MR antigen levels compared with those in non-treated control macrophages, indicating a down-regulation of MR antigen expression by antisense ODN.

Cytokine and chemokine mRNA levels in macrophages treated with antisense ODN and *C. albicans*. Cytokine and chemokine mRNA levels in macrophages following *C. albicans* attachment were compared in untreated macrophages and in macrophages

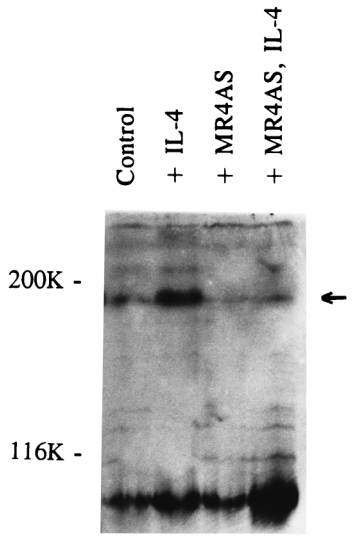

FIG. 4. Western blot analysis of MR in antisense ODN- and/or IL-4-treated macrophages. Macrophages were treated with 100 nM antisense ODN (MR4AS) for 3 days and/or 5 ng of IL-4 per ml for 24 h as described in Materials and Methods. Macrophage membrane fractions were analyzed by ECL Western blotting with anti-MR antibody. The numbers on the left refer to molecular mass standards (in daltons). The arrow indicates the position of MR.

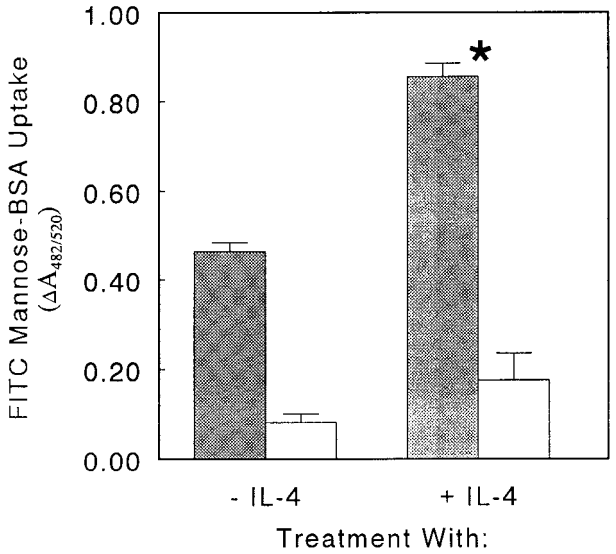

FIG. 6. Effect of IL-4 treatment on FITC-labeled mannose-BSA uptake by macrophages. Macrophages were treated with 5 ng of IL-4 per ml for 24 h at 37°C. Uptake of FITC-labeled mannose-BSA was determined in the presence (□) or absence (▨) of 0.1 M αMM. Each bar represents the mean (error bar indicates ± standard error) for three macrophage cultures. *, P of <0.05 compared with untreated macrophage group.

in which MR expression had been down-regulated by treatment with antisense ODN. As shown in Fig. 5, the reduction of MR expression resulted in a significant reduction of cytokine mRNA elevations for IL-1β, IL-6, and GM-CSF. In contrast, the levels of chemokine mRNAs for MIP-1β, MIP-2, and KC were not affected by the reduced MR expression levels. These results indicate that specific cytokines may be induced through macrophage MRs bound by *C. albicans*, but induction of some chemokine mRNA synthesis may be mediated by other receptors.

Effect of IL-4 on cytokine and chemokine mRNA inductions by *C. albicans* attachment. In order to confirm the involvement of MRs in the elevation of cytokine mRNA levels by *C. albicans* attachment, the possible alteration of cytokine mRNA levels by MR expression was examined. For this purpose IL-4, which has been reported to enhance MR activity in cultured macrophages (28), was used. Figure 4 shows increased MR expression, which can be suppressed by antisense ODN treatment, in IL-4-treated macrophages as demonstrated by Western blotting. Similar results were also observed in an FITC-mannose-BSA uptake assay (Fig. 6). That is, IL-4 treatment significantly enhanced the uptake of FITC-mannose-BSA at 37°C, but it was still inhibited by the presence of αMM. Uptake of either FITC-BSA or FITC-glucose-BSA was not enhanced by IL-4 treatment (data not shown). Thus, up-regulation of MR by IL-4 treatment was evident by functional as well as antigen expression level assays. GM-CSF mRNA and MIP-2 mRNA expression following *C. albicans* attachment to macrophages in which MR expression had been up-regulated was examined. As shown in Fig. 7, up-regulation of MR expression by IL-4 markedly enhanced the elevation of GM-CSF mRNA in response to *C. albicans* attachment. The chemokine MIP-2 response was not altered by the up-regulation of MR expression.

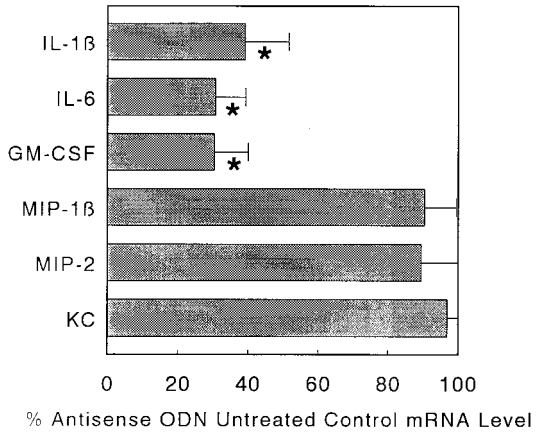

FIG. 5. Levels of cytokine and chemokine mRNAs in macrophages treated with antisense ODN and *C. albicans*. Macrophages were treated first with 100 nM antisense ODN for 3 days and then with CyD for 30 min and finally were incubated with *C. albicans* for 1 h in the presence of CyD. Levels of mRNA (percentage of that in antisense control [with no ODN treatment]) were calculated as follows: (level of mRNA in antisense ODN-treated macrophages/level of mRNA in antisense untreated control macrophages) × 100. *, P of <0.05 compared with antisense control group (no ODN treatment).

DISCUSSION

It has been widely accepted that the first line of defense to *C. albicans* is neutrophils, which can ingest and kill yeast cells (1). In contrast, macrophages appear less efficient in killing yeasts. However, macrophages secret a variety of proinflammatory cytokines, including chemokines, which regulate inflammatory responses and contribute to the generation of acquired immunity to microbial infection. Therefore, mechanisms of cytokine and chemokine induction by macrophages following interaction with *C. albicans* are important in understanding the disease process. The cytokine GM-CSF, which was studied in this report as a representative cytokine, is a potent activator of macrophages and induces differentiation of precursor cells as well as anti-*C. albicans* activity (13, 20, 31). MIP-2 was used as a representative chemokine in this study, since it is a mem-

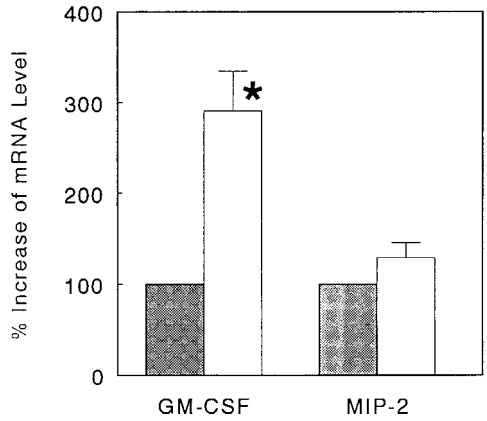

FIG. 7. Effect of IL-4 treatment on cytokine GM-CSF and chemokine MIP-2 mRNA inductions by *C. albicans* attachment. Macrophages were treated first with 5 ng of IL-4 per ml for 24 h and then with CyD for 30 min and finally were incubated with *C. albicans* for 1 h in the presence of CyD. ■, untreated; □, IL-4 treated; *, *P* of <0.05 compared with untreated macrophage group.

ber of the α chemokine subfamily that causes marked neutrophil chemotaxis and activation (16, 33). Both GM-CSF and MIP-2 are produced by macrophages and may contribute to the control of *C. albicans*, but the details are not fully understood.

In present study we showed that attachment of *C. albicans* to macrophages in the absence of ingestion, achieved by using CyD and a short incubation time to avoid indirect induction of cytokines (34), was sufficient to generate a signal for induction of increased steady-state levels of certain cytokine and chemokine messages. The data are similar to those obtained with other microorganisms reported previously by us (34). Therefore, interactions of certain microbial surface ligands and their corresponding receptors on macrophages appear important for the induction of mRNA synthesis for selected cytokines and chemokines. Others have shown that opsonized microbes may be recognized by macrophages through complement receptors, such as CR1, CR2, and CR3, or receptors to immunoglobulin, such as Fc receptors, and that these receptors appear to be involved in the induction of cytokine synthesis (37). Polymerized C3b and C3b-Sepharose can also induce cytokine synthesis by macrophages (3). However, complement-mediated recognition obviously requires the presence of complement, and macrophages in an environment with less abundant serum components, such as alveolar spaces, are not able to utilize complement or Fc receptors for recognition of microbes (10). A role for a glucan-binding site on complement receptor CR3 has been suggested as well (25). However, ligand-binding studies with purified CR3 showed no binding by this complement receptor to unopsonized zymosan particles or to soluble β-glucan (30), and functional studies with monocytes from patients genetically deficient in CR3 showed no impairment in the zymosan-induced production of platelet-activating factor by a mechanism inhibitable by soluble β-glucan (8).

Mannan is a major component of *Candida* cell walls and may be recognized by macrophages for phagocytosis of yeasts (17, 18). The possible involvement of MR in phagocytosis of *C. albicans* by macrophages seems logical due to the high affinity of MR to mannan, but there is no direct evidence. On the other hand, immunological responses of macrophages to *C. albicans*, such as cytokine and chemokine responses, may or may not require ligand-receptor interactions different from those of phagocytosis, since phagocytosis involves mechanical dynamics but an immunological response does not. Inhibition of *C. albicans*-macrophage interaction by αMM regarding elevation of cytokine and chemokine mRNA levels as shown in the present study indicates the involvement of αMM-sensitive ligand-receptor interactions in the cytokine GM-CSF response of macrophages to *C. albicans* attachment, suggesting a possible MR involvement. However, chemokine MIP-2 induction was not affected by αMM, suggesting that an αMM-insensitive ligand receptor might be involved. Thus, these results indicate the possibility that certain cytokine and chemokine responses of macrophages to *C. albicans* may be regulated by different receptor groups.

The use of antisense ODNs in cultured cells is a powerful strategy for analysis of receptor involvement. Since antisense ODNs can specifically inhibit target protein expression without any effect on the expression of other receptors, their use is suitable for analysis of the involvement of MR in *C. albicans*-macrophage interactions. The treatment of macrophages with the antisense ODN designed to hybridize to the 3′-untranslated region of MR mRNA showed a significant inhibition of both expression and function of MR, as determined by Western blotting and ^{125}I-mannose-BSA binding assays. By use of such MR-down-regulated macrophages, cytokine and chemokine responses to *C. albicans* attachment were compared with those of antisense-ODN-untreated macrophages. The elevation of cytokine mRNAs (IL-1β, IL-6, and GM-CSF) caused by *C. albicans* attachment to the macrophages was significantly reduced in MR-down-regulated macrophages, but the elevation of chemokine (MIP-1β, MIP-2, and KC) mRNA levels was not affected. These results indicate that not only GM-CSF but also other cytokines, such as IL-1β and IL-6, may be induced through MRs. That is, a certain group of cytokine genes might be regulated by MRs during *C. albicans* attachment to macrophages, but chemokines are separately regulated by other receptors. This explanation was further confirmed by experiments with MR-up-regulated macrophages.

IL-4 treatment is known to up-regulate MR activity as determined by ^{125}I-mannose-BSA binding assays as well as RT-PCR tests for MR mRNA (28). Our study further confirmed the up-regulation of MR expression by IL-4 treatment with both functional (FITC-mannose-BSA uptake assay) and antigen level (Western blot) assays. The elevation of cytokine GM-CSF mRNA levels by *C. albicans* attachment was further enhanced by the MR up-regulation induced by IL-4 treatment, but there was no significant enhancement of the elevation of chemokine MIP-2 mRNA levels. Even though IL-4 treatment may not enhance solely MR expression by macrophages, the enhancement of GM-CSF induction, but not MIP-2 induction, adds further evidence supporting the involvement of MR in cytokine GM-CSF induction by *C. albicans* attachment.

In summary, *C. albicans* attachment to macrophages was recognized by macrophage MRs and resulted in elevation of message levels of certain cytokines, such as IL-1β, IL-6, and GM-CSF. Chemokine messages, such as MIP-1β, MIP-2, and KC, were also elevated by *C. albicans* attachment, but different receptors may be involved. Thus, cytokine and chemokine responses of macrophages to *C. albicans* may be regulated by different receptor groups.

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