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Specific Recognition of *Candida albicans* by Macrophages Requires Galectin-3 to Discriminate *Saccharomyces cerevisiae* and Needs Association with TLR2 for Signaling[1]

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Stimulation of cells of the macrophage lineage is a crucial step in the sensing of yeasts by the immune system. Glycans present in both *Candida albicans* and *Saccharomyces cerevisiae* cell walls have been shown to act as ligands for different receptors leading to different stimulating pathways, some of which need receptor co-involvement. However, among these ligand-receptor couples, none has been shown to discriminate the pathogenic yeast *C. albicans*. We explored the role of galectin-3, which binds *C. albicans* β-1,2 mannosides. These glycans are specifically and prominently expressed at the surface of *C. albicans* but not on *S. cerevisiae*. Using a mouse cell line and galectin-3-deleted cells from knockout mice, we demonstrated a specific enhancement of the cellular response to *C. albicans* compared with *S. cerevisiae*, which depended on galectin-3 expression. However, galectin-3 was not required for recognition and endocytosis of yeasts. In contrast, using PMA-induced differentiated THP-1, we observed that the presence of TLR2 was required for efficient uptake and endocytosis of both *C. albicans* and *S. cerevisiae*. TLR2 and galectin-3, which are expressed at the level of phagosomes containing *C. albicans*, were shown to be associated in differentiated macrophages after incubation with this sole species. These data suggest that macrophages differently sense *C. albicans* and *S. cerevisiae* through a mechanism involving TLR2 and galectin-3, which probably associate for binding of ligands expressing β-1,2 mannosides specific to the *C. albicans* cell wall surface. *The Journal of Immunology,* 2006, 177: 4679–4687.

Host sensing of microbial exposure is conferred by the expression of extracellular and intracellular pathogen-recognition molecules (PRMs).[3] Upon recognition of pathogen-associated molecular patterns (PAMPs) by PRMs, specific intracellular signaling pathways modulate essential host defense functions such as inflammation, cell death, and adaptive immunity. However, whereas the function of the membrane-bound PRMs known as TLRs has been studied extensively over the past few years (1), lectins have also been long established to confer an extracellular surveillance mechanism promoting phagocytosis and signaling (2, 3).

Candida albicans is one of the most prominent human pathogens, and also colonizes the mucocutaneous surfaces of the oral cavity, gastrointestinal tract and vagina. Given the high levels of morbidity and mortality induced by *C. albicans* in hospitalized patients, the pathogenic mechanisms of *C. albicans* have been investigated extensively (4). However, unlike *Drosophila melanogaster*, which provides a model for the sensing of indigenous and/or pathogenic fungi, the nature of the mammalian PRMs conferring discrimination between the large number of fungal species closely associated with different aspects of human life remains poorly understood.

The complex architecture of the yeast cell wall is composed of chitin, glucans, mannoproteins, mannan, and glycolipids. The protective and inflammatory response to fungal exposure is initiated by host recognition of specific PAMPs, which are expressed by pathogenic and/or symbiotic microorganisms (5). Notably, *C. albicans* and *Saccharomyces cerevisiae* can both lead to TLR-dependent proinflammatory cytokine production (6, 7). Alternatively, certain lectins, such as dectin-1, have been proposed to recognize surface sugars of microorganisms and to cooperate with TLRs for induction of a proinflammatory response (8). Notably, dectin-1 cooperates with TLR2 to confer cellular responsiveness to microbial β-glucans (9–11). Association has also been demonstrated for SIGNR1, another lectin involved in yeast recognition (12, 13), which enhances TLR4/MD2-induced signaling by LPS (14). It therefore seems that lectins may act in specific recognition by TLRs, and enhance signals induced through these receptors.

Unlike β-glucans and α-mannan, which are expressed by both nonpathogenic and pathogenic yeasts, host recognition of β-1,2 mannosides predicts the host sensing of *C. albicans* (15). β-1,2 mannosides are special types of glycans that are expressed by *C. albicans* and are associated with both mannan and phospholipomannan (PLM) (16), a glycolipid present in the cell wall (17) that binds to the macrophage membrane (18) and leads to TLR2-dependent NF-κB activation and TNF-α production (19). Purified β-1,2 mannosides have been shown to elicit TNF-α (20) and eicosanoid (21) production. However, in contrast to other fungal mannosides,

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[3] Abbreviations used in this paper: PRM, pathogen-recognition molecule; PAMP, pathogen-associated molecular pattern; PLM, phospholipomannan; KO, knockout; WT, wild type; DC-SIGN, dendritic cell-specific ICAM-3 grabbing nonintegrin; SIGNR1, a murine homolog of human DC-SIGN.

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which react with C-lectins, such as the mannose receptor (22) or DC-SIGN/SIGNR1 (12), β-1,2 mannosides bind to a host 32-kDa protein, identified as the S-lectin galectin-3 (23). In this study, we investigated the physiological role of galectin-3 in the immune response to *C. albicans*. Our results revealed that galectin-3, but not TLR2, is dispensable for the recognition and endocytosis of yeasts, but that galectin-3 is essential for TLR2-dependent cytokine production in response to *C. albicans* but not *S. cerevisiae*.

Materials and Methods

Reagents and Abs

All reagents were obtained from Sigma-Aldrich. The TLR2 agonist Pam_3CSK_4 was from EMC Microcollections. The anti-β-1,2 mannoside mAb 5B2, a rat IgM, was developed in our laboratory (24). Anti-β-1,3 glucan, a mouse monoclonal IgG, was provided from Biosupplies. Anti-human/mouse TLR2 Ab (clone TL2.5) was obtained from eBioscience. The human recombinant galectin-3 and polyclonal rabbit anti-human galectin-3 Abs were a gift from F. van den Brule, University of Liège (Liège, Belgium). mAbs to mouse or human galectin-3 (Novacastra Laboratories) were also used depending on the experiment. HRP-, FITC-, and PE-conjugated anti-mouse or anti-rabbit IgG or IgM were obtained from Southern Biotechnology Associates. Fluorescent phallotoxin (BODIPY FL phallacidin) was purchased from Molecular Probes (Interchim).

Cell lines

The mouse macrophage-like cell line J774 (ECCC 85011428) was derived from a tumor of a female BALB/c mouse. Adherent cells were cultured at 37°C in an atmosphere containing 5% CO_2 in DMEM supplemented with 10% heat-inactivated FCS (Valbiotech), 5 mM L-glutamine, 100 μg/ml streptomycin, and 50 μg/ml penicillin (culture medium). Before use, cells were gently scraped off with a rubber policeman and, depending on the experiment, either plated into eight-well Lab-Tek tissue culture chambers (Nunc) or into 48-well tissue culture dishes at a concentration of 5×10^5 cells/well.

Human monocyte THP-1 cells (ATCC TIB 202) were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated FCS. Cell differentiation was induced by treatment with different doses of PMA in culture medium. After 20 h incubation, the cells were washed and cultured for 12 h without PMA. With this treatment, cells acquired the morphologic characteristics of macrophages.

Isolation of peritoneal macrophages

The generation of galectin-$3^{-/-}$ mice by gene-targeting technology has been described elsewhere (25). As controls, age- and sex-matched wild-type (WT) littermate mice were used. Mice were maintained for us by Charles River Breeding Laboratories. Mouse peritoneal macrophages were obtained from 6- to 8-wk-old WT or galectin-3 knockout (KO) mice by injecting 2 ml of PBS containing 10% proteose peptone broth. After 4 days, the mice were killed and peritoneal macrophages were harvested by injecting 4 ml of sterile DMEM. After centrifugation and washing, the cells were resuspended in culture medium and cultured for 24 h in 75-cm^2 culture flasks. For coculture experiments, adherent cells were recovered by gentle scraping and transferred into 96-well microtiter plates at 5×10^5 cells/well, in a final volume of 200 μl of culture medium.

Yeast culture

C. albicans (serotype A) and *S. cerevisiae* were maintained on Sabouraud dextrose agar at 4°C. Before the experiments, yeast cells were transferred onto fresh Sabouraud dextrose agar and incubated for 20 h at 37°C. Yeast cells were then recovered, washed with PBS, and transferred into DMEM. Three different strains of *C. albicans* (SC5314, the derived strain CAF and VW32, which was a strain isolated in our laboratory) were used. The S288C strain of *S. cerevisiae* was used as a reference. In some experiments, two additional strains derived from S288C were tested (S288C-1 and S288C-4).

Cytokine production by mammalian cells after stimulation by yeasts

Plated cells were incubated at 37°C with yeasts at a concentration of five yeasts per cell. Supernatants were collected after different periods of time and stored at −20°C until cytokine assays were performed. TNF-α concentrations in cell-free supernatants were measured with commercial ELISA kits according to the manufacturer's instructions (R&D Systems). In some experiments, TNF-α production was also evaluated by RT-PCR (discussed below).

Microscopic examination and immunofluorescent staining

Yeasts were incubated with 100 μl of human recombinant galectin-3 in PBS for 2 h at 20°C. After washing, bound galectin-3 was revealed with a 1/100 dilution of rabbit polyclonal Ab specific for human galectin-3 previously adsorbed onto yeasts. After washing, 100 μl of a 1/100 dilution of FITC-conjugated goat anti-rabbit IgG in phosphate buffer was added for 1 h at 20°C. Slides were then washed and mounted for microscopic examination.

Expression of galectin-3 and TLR2 was evaluated on J774 and THP-1 cells previously plated into Lab-Tek wells in the absence of yeasts or after coculture of cells with yeasts for 2 h at 37°C. Cells were washed with warm DMEM, fixed and permeabilized with a mixture of 3.7% formaldehyde and 0.2% Triton X-100 in phosphate buffer at 20°C for 20 min. One hundred microliters of a 1/100 dilution of anti-galectin-3- or anti-TLR2-specific mAb was added for 2 h at 20°C. After five washes, 100 μl of a 1/100 dilution of FITC-conjugated goat anti-mouse IgG in phosphate buffer was added for 1 h at 20°C. In parallel, F-actin labeling was performed by incubating permeabilized cells with a 1/100 dilution of BODIPY FL phallacidin for 20 min at 20°C. Slides were then washed five times and mounted for microscopic examination.

Interaction of recombinant galectin-3 with C. albicans

Different concentrations of human recombinant galectin-3 in PBS were coated into 96-well culture plates for 1 h at 20°C followed by overnight incubation at 4°C. Plates were saturated with 5% BSA in PBS for 2 h at 20°C. After washing, 200 μl of yeast suspension was added to the wells and incubated for 2 h at 20°C. Unbound yeasts were discarded by washing with PBS containing 0.5% BSA and bound yeasts were revealed using the mAb EBCA1 directed against *C. albicans* mannan (26) and anti-mouse IgM FITC-conjugated Ab. Plates were read in a Fluorocount reader (Packard).

Flow cytometry analysis

The expression of β-1,2 mannosides and β-1,3 glucans on the yeast cell wall was studied by flow cytometry. For preparation of SC5314 heat-killed yeasts, the cells were incubated for 20 min at 90°C and washed once in PBS. For staining, 10^6 cells were washed with PBS containing 2% FCS and then incubated for 15 min with mAb 5B2 to β-1,2 mannosides, diluted 1/200, or with a mAb to β-1,3 glucans diluted 1/50. For Ab detection, cells were incubated for 15 min with FITC-labeled anti-rat IgM or PE-labeled anti-mouse IgG second Ab diluted 1/100. A negative control was performed by adding labeled second Ab at the same concentration. All processes were conducted at 4°C. After washing, cells were fixed in 0.4% paraformaldehyde and analyzed with a FACS.

Flow cytometry was performed with an EPICS XLMCL4 (Beckman Coulter) equipped with an argon ion laser with an excitation power of 15 mW at 488 nm. Forward and side light scatter were analyzed on linear scales, while green and red fluorescence intensity was determined on logarithmic scales. Analysis gates were set around debris and intact cells were set on a forward and side light scatter dot plot. The fluorescence histograms of 5000 cells were generated using gated data. Data acquisition and analysis were performed using WINMDI software (available from ⟨http://facs.scripps.edu⟩). Mean fluorescence intensities were obtained by subtracting values for negative controls from the values given by each Ag.

Immunoprecipitation and Western blot analysis

For total extraction of proteins, macrophages (10^6 cells) were treated by boiling in 500 μl of 2× concentrated electrophoresis sample 1× buffer (125 mM Tris-HCl (pH 6.8), 2% SDS, 5% glycerol, 1% 2-ME, and bromphenol blue). Lysates were clarified by centrifugation for 10 min at 12,000 × *g* at 4°C. For immunoprecipitation, 10^7 cells were extracted with 500 μl of RIPA buffer (PBS containing 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, PMSF, aprotinin) for 30 min on ice. Lysates were collected and clarified by centrifugation for 10 min at 12,000 × *g* at 4°C. Supernatants (500 μl) were incubated with either anti-galectin-3 or anti-TLR2 Ab for 2 h at 4°C. Protein G-coupled agarose beads (10 μl) were added to each tube and incubated for 18 h at 4°C. Beads were washed 5 to 10 times with PBS. Precipitates were recovered by addition of 50 μl of sample buffer and boiled for 10 min at 100°C.

Extracted proteins or precipitates were separated by 10% SDS-PAGE before blotting onto a nitrocellulose membrane (Schleicher & Schuell) for 2 h at 200 mA in a semidry transfer system. After staining with 0.1% Ponceau S in 5% acetic acid to confirm equivalence of loading and transfer, the membrane was blocked by incubation with TNT buffer (10 mM Tris, 100 mM NaCl, and 0.1% Tween 20) containing 5% nonfat dry milk for 1 h at 20°C. Membranes were probed with anti-galectin-3 or anti-TLR2 Abs (diluted 1/1000) in TNT-5% BSA for 2 h at 20°C. After washing several times, the membranes were incubated for 1 h at 20°C with a 1/2000 dilution

of HRP-conjugated anti-mouse IgG in TNT-5% BSA. After washing, the membrane was incubated with ECL detection reagents (SuperSignal Chemiluminescent substrate; Pierce) and exposed to Hyperfilm ECL.

RT-PCR

THP-1 cells (10^7) treated with different concentrations of PMA and incubated with or without *C. albicans* blastoconidia were washed with PBS and total RNA was extracted with an RNeasy Mini kit (Qiagen). The reverse transcription reaction was performed using Superscript II reverse transcriptase (Invitrogen Life Technologies) at 42°C for 60 min in the presence of 2 U of RNase inhibitor. Following reverse transcription, the enzyme was heat-inactivated at 99°C for 3 min. PCR was performed in an iCycler (Bio-Rad) with the primers designed using Primer 3 software from gene accession nos. NM002306, U88878, and AF043342 for human galectin-3, TLR2, and TNF-α, respectively. Control genes used were GAPDH or 28S rRNA. The following primers synthesized by Eurogentec were used for fragment amplification: galectin-3 302 bp, forward (353–373) 5′-GGCCACTGATTGTGCCTTAT-3′ and reverse (654–674) 5′-TGCAACCTTGAAGTGGTCAG-3′; TLR2 311 bp, forward (2162–2182) 5′-ACTTCATTCCTGGCAAGTGG-3′ and reverse (2472–2492) 5′-CGCAGCTCTCAGATTTACCC-3′; and TNF-α 335 bp, forward (419–439) 5′-AGCCCATGTTGTAGCAAACC-3′ and reverse (753–773) 5′-GGAAGACCCCTCCCAGATAG-3′.

The PCR conditions used to amplify fragments were: 30 cycles at 94°C for 1 min, 60°C for 1 min, and 72°C for 45 s. Hot start at 94°C was performed using AmpliTaq Gold (Applied Biosystems). Products were analyzed by electrophoresis and staining with ethidium bromide. Semiquantification was performed after scanning with GelEval (FrogDance software).

Statistics

For cytokine determination, results of duplicate measurements (expressed as mean ± SD) from a representative experiment performed at least twice are shown. Significance of the data was evaluated by ANOVA. Other results were from three to five independent experiments.

Results

C. albicans*, but not* S. cerevisiae*, expresses β-1,2 mannosides*

β-1,2 mannosides have been previously shown to be essential for the binding of *C. albicans* to macrophages and to be recognized by a 32-kDa protein homologous to galectin-3 (15, 23). Using flow cytometry with specific mAbs, we determined and compared the yeasts surface expression of β-1,3 glucans with β-1,2 mannosides. Unlike β-1,3 glucans, which were accessible only after heat treatment of *C. albicans*, β-1,2 mannosides were constitutively expressed at the surface of both heat-killed and live *Candida* yeasts (Fig. 1). Furthermore, *S. cerevisiae* did not express β-1,2 mannosides (Fig. 1), suggesting that galectin-3 might contribute to the sensing of *C. albicans*.

Galectin-3 binds C. albicans

To begin to understand the potential role of galectin-3 in the immune response to *C. albicans*, we conducted experiments to

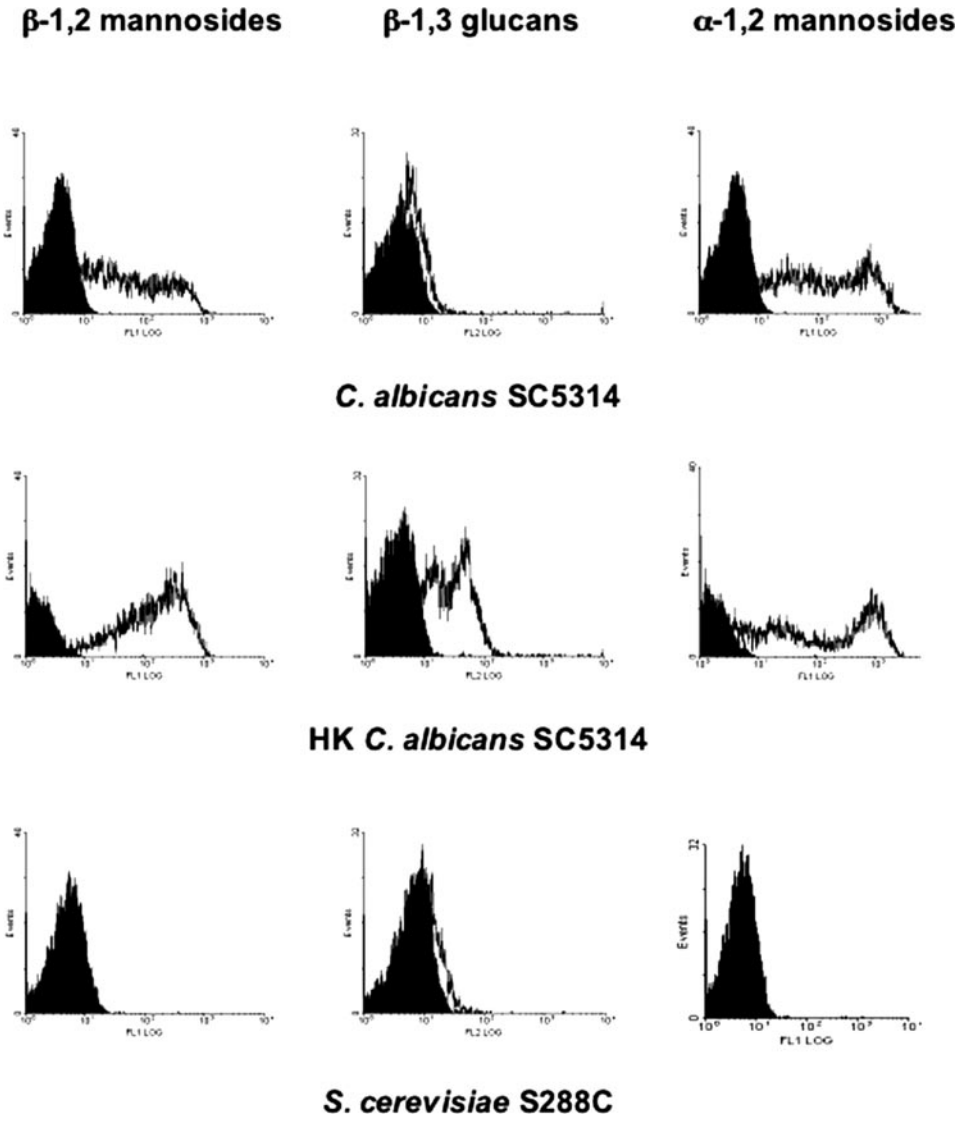

FIGURE 1. Surface expression of β-1,2 mannosides and β-1,3 glucans by *C. albicans* and *S. cerevisiae*. Flow cytometry was performed with mAbs to α-mannoside or β-1,2 mannosides and β-1,3 glucans on live or heat-killed (HK) *C. albicans* strain SC5314 and compared with live *S. cerevisiae* strain S288C. The results presented are representative of three independent experiments. Gray-filled histogram represents negative control without primary Ab.

confirm the interaction of galectin-3 with *C. albicans* blastoconidia. *C. albicans* yeast cells were added to wells of microtiter plates coated with different concentrations of human recombinant galectin-3. Using an anti-mannan Ab, we revealed a dose-dependent binding of *C. albicans* to human recombinant galectin-3 (Fig. 2*A*). Furthermore, using a specific Ab to galectin-3, human recombinant galectin-3 was detected at the surface of *C. albicans* (Fig. 2*B*). Conversely, we failed to detect any binding of *S. cerevisiae* to human recombinant galectin-3 (data not shown).

Galectin-3 is localized in phagocytic cups and phagosomes containing C. albicans

To determine the localization of galectin-3 in macrophages engaged in phagocytosis of *C. albicans* blastoconidia, immunofluorescent microscopic analysis was performed using the mouse macrophage line J774. Galectin-3 expression was assessed in cells incubated with or without yeasts. As shown previously (27), entry of *C. albicans* into macrophages was associated with the condensation of actin filaments as revealed by the labeling of F-actin around ingested yeasts (Fig. 3, *A* and *B*). Parallel staining of galectin-3 revealed that after incubation with *C. albicans*, the lectin also localized around the yeasts (Fig. 3, *C* and *D*) either at the cell membrane at the level of phagocytic cups or within the cells (Fig. 3*E*).

Higher amounts of TNF-α are produced after C. albicans *stimulation of J774 cells*

Because galectin-3 may play a role in the specific recognition of *C. albicans* compared with *S. cerevisiae*, the behavior of J774 cells toward both yeasts was examined, particularly whether the yeasts were recognized and engulfed by the cells. J774 cells were able to bind and endocytose both yeast species in a similar manner (Fig. 4*A*). The ability of the three strains of *C. albicans* and *S. cerevisiae* to stimulate macrophages was determined and compared (Fig. 4*B*). Unexpectedly, *C. albicans* induced a 2.5- to 3-fold higher TNF-α production compared with *S. cerevisiae*-stimulated cells, suggesting that *C. albicans* is somehow more efficient at triggering the proinflammatory response.

Galectin-3 deletion does not alter the ability of macrophages to recognize and endocytose C. albicans *and* S. cerevisiae

The role of galectin-3 in the enhancement of cell stimulation observed with *C. albicans* was then examined using macrophages isolated from either WT or galectin-3 KO mice. Absence of galectin-3 expression in macrophages from KO mice was first verified by Western blotting (Fig. 5*A*). The effect of deletion of the galectin-3 gene on the phagocytic capacity of macrophages toward the yeasts was then analyzed. As shown in Fig. 5*B*, both yeast

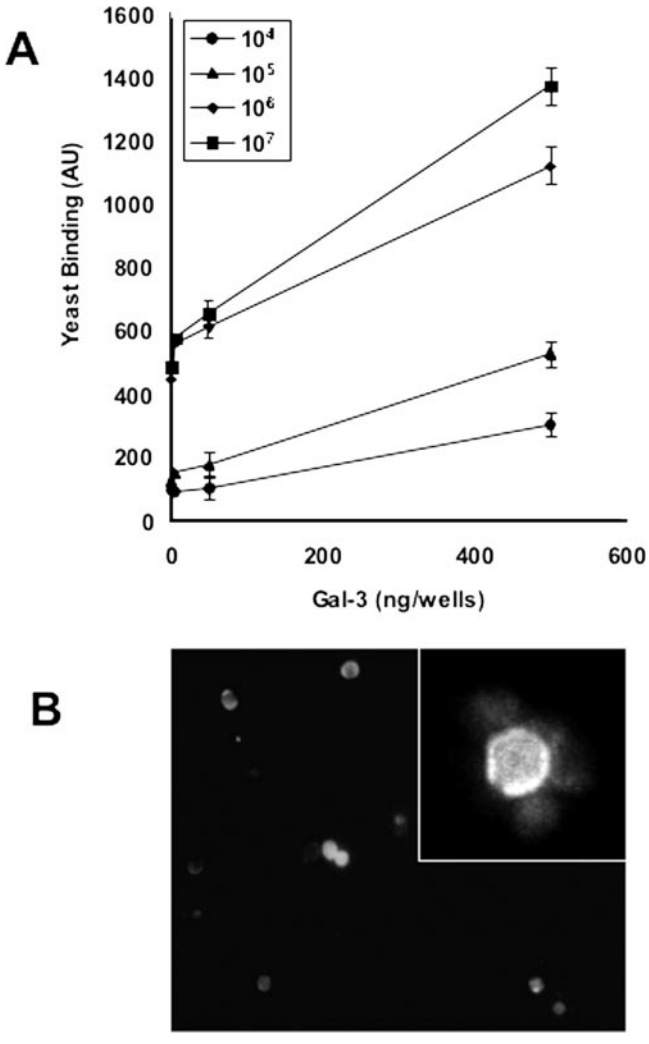

FIGURE 2. Binding of galectin-3 to *C. albicans* blastoconidia. *A*, Binding of yeasts to galectin-3 was evaluated by cyto-ELISA. After coating wells with different concentrations of galectin-3 (Gal-3), different amounts of *C. albicans* blastoconidia were added and bound yeasts were revealed with FITC-conjugated anti-*C. albicans* mannan mAb. Fluorescence of duplicate wells was measured in a fluorocount. *B*, Binding of human recombinant galectin-3 to blastoconidia was examined by indirect immunofluorescence using an anti-galectin-3 polyclonal Ab to reveal bound human recombinant galectin-3. Detail of a labeled yeast (*inset*) is shown.

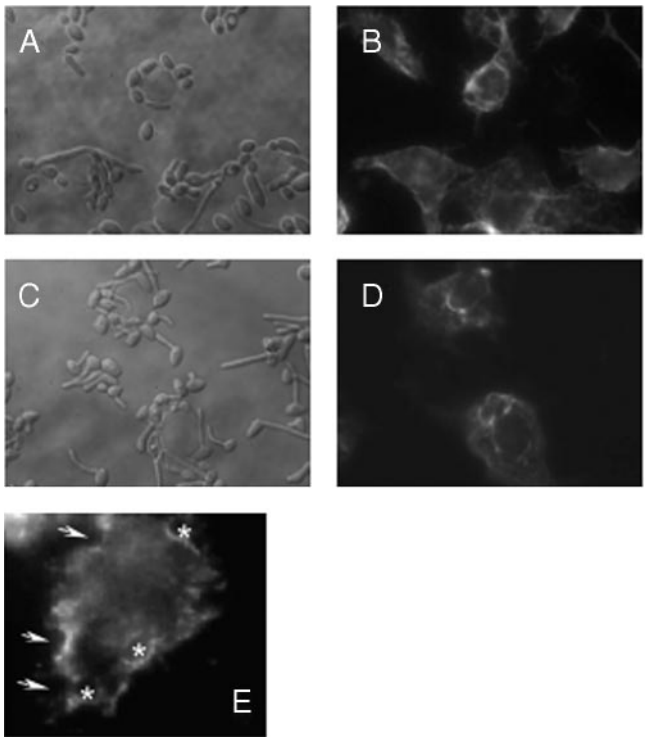

FIGURE 3. Localization of galectin-3 in J774 cells containing engulfed *C. albicans* blastoconidia. After coculture of J774 cells with yeasts at a yeast to cell ratio of 5:1, F-actin (*A* and *B*), and galectin-3 (*C–E*) distributions were determined using FITC-conjugated phallacidin and an anti-galectin-3 mAb, respectively. *B* and *D*, Corresponding phase-contrast images of fluorescence examination (*A* and *C*). *E*, Details of galectin-3 labeling show the arrows indicate phagocytic cups formed around the yeasts. Engulfed yeasts (∗) are represented.

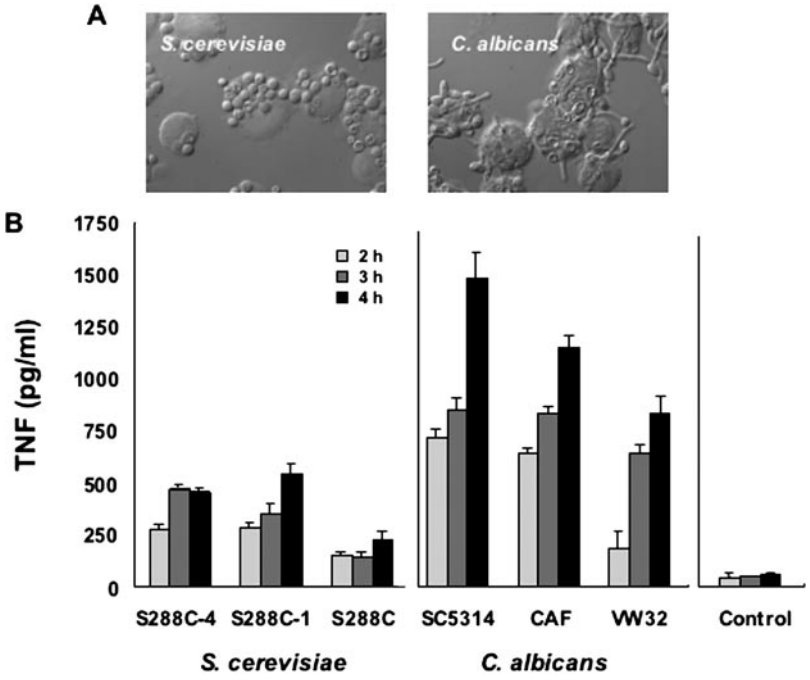

FIGURE 4. Comparison of interaction of J774 cells with *C. albicans* and *S. cerevisiae*. *A*, J774 cells were incubated with either *C. albicans* strain SC5314 or *S. cerevisiae* strain S288C. After washing, cocultures were examined by phase-contrast microscopy. *B*, Stimulation of J774 cells by yeasts was evaluated by measuring the induction of TNF-α production after coculture of cells with three strains of either *C. albicans* or *S. cerevisiae* for different time points. Cytokine production was measured in cell-free supernatants by ELISA. Data are expressed as the mean $\pm$ SD of cytokine concentration and are from one representative among three experiments performed.

strains were recognized by macrophages isolated from either WT or galectin-3 KO mice. This result shows that deletion of galectin-3 does not qualitatively alter the binding capacity of the macrophages toward yeasts.

Specific induction of cytokines by C. albicans *depends on galectin-3 expression by the cells*

The effect of galectin-3 deficiency on the capacity of macrophages to respond to both *C. albicans* and *S. cerevisiae* was then determined. To this end, cells were incubated with either *S. cerevisiae* or *C. albicans* blastoconidia and the production of TNF-α was measured. With macrophages from WT mice, *C. albicans* induced a significantly ($p < 0.05$, $n = 4$) higher production of the cytokine (1424 $\pm$ 141 pg/ml) compared with that obtained with *S. cerevisiae* (352 $\pm$ 103 pg/ml). These results, obtained with primary macrophages from WT mice, confirm those obtained with J774 cells (as discussed). In contrast, when macrophages from galectin-3 KO mice were used in the experiment, no significant difference in TNF-α production was observed. In this case, the amount of cytokine obtained after incubation with *C. albicans* (366 $\pm$ 24 pg/ml) was similar to that observed with *S. cerevisiae* (241 $\pm$ 56 pg/ml). Similar results were observed whatever strain of *C. albicans* was used to stimulate the cells (Fig. 6*B*). To ascertain that this effect is not due to intrinsic defect of galectin-3-deficient cells to produce cytokines in response to TLR2, cells were stimulated with the specific TLR2 agonist Pam_3CSK_4. As observed in Fig. 6*C*, no differences were noticed between galectin-3-competent and galectin-3-deficient cells. Altogether, this indicates that the specific response of macrophages to *C. albicans* is due, at least in part, to galectin-3.

PMA differentiates THP-1 cells and induces galectin-3 and TLR2 expression

Human monocytic THP-1 cells, which are known to differentiate into macrophages after treatment with PMA, were used to examine the role of both receptors in the interaction of macrophages with yeasts. THP-1 cells were treated with different doses of PMA and maturation was followed according to adherence, morphology, and expression of galectin-3 and TLR2. Microscopic examination and fluorescence analysis (Fig. 7*A*) revealed that PMA treatment led to differentiation from a monocyte-like to a macrophage-like morphology and adherence. Immunofluorescence showed that both galectin-3 and TLR2 membrane expression was induced by PMA. Similarly, when galectin-3 gene expression was evaluated (Fig. 7*B*), a relationship between the amount of mRNA and the degree of cell maturation was observed, a maximum being obtained when cells had been treated with 10 ng/ml PMA. For TLR2, a lower dose of PMA was needed to obtain the maximum amount of mRNA. This increased significantly and reached a maximum with 1 ng/ml PMA. According to the results obtained by immunofluorescence, Western blotting of the corresponding proteins showed that TLR2 was absent and galectin-3 was present in undifferentiated cells (Fig. 7*C*). Expression of both proteins was increased after treatment of cells with 10 ng/ml PMA (Fig. 7*C*).

Differentiated THP-1 cells express TLR2 associated with C. albicans*-containing phagosomes and produce TNF-α*

The effect of maturation on the ability of THP-1 cells to interact with *C. albicans* was investigated. Binding of blastoconidia to undifferentiated THP-1 cells was limited (Fig. 8*A*, *a*). In contrast, PMA-treated THP-1 cells (Fig. 8*A*, *b*) bound and endocytosed *C. albicans* in a manner that was similar to mouse primary macrophages (Fig. 5*B*) or J774 cells (Fig. 4*A*). With these cells, TLR2 could be localized at the level of engulfed yeasts (Fig. 8*A*, *c* and *d*). The ability of differentiated THP-1 cells, which express TLR2 and phagocytose yeasts, to respond to *C. albicans* was then investigated. In contrast to immature cells that do not produce TNF-α in response to *C. albicans*, after PMA treatment a high amount of mRNA was induced by yeasts in the cells (Fig. 8*B*).

TLR2 and galectin-3 associate when differentiated THP-1 cells are in contact with C. albicans

As suggested by our experiments, precipitation with their respective specific Abs demonstrated that both TLR2 and galectin-3 were increasingly present in macrophage extracts as a function of the PMA dose inducing THP-1 differentiation (Fig. 7). Whether an

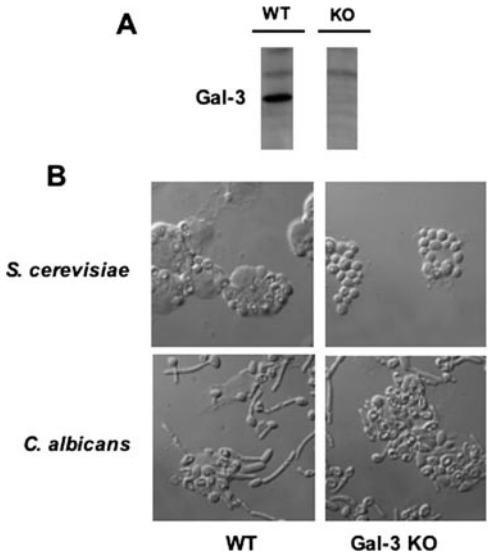

FIGURE 5. Galectin-3 is not necessary for binding and endocytosis of either *C. albicans* or *S. cerevisiae* blastoconidia. *A*, Expression of galectin-3 by peritoneal macrophages from galectin-3 KO or WT mice revealed by Western blotting using a mAb to galectin-3. *B*, Examination by phase-contrast microscopy of endocytosis of *C. albicans* and *S. cerevisiae* yeasts by macrophages from WT or galectin-3 (Gal-3) KO mice.

association of TLR2 and galectin-3 appeared in the presence of *C. albicans* was therefore explored through experiments based on coprecipitation with Abs to both proteins. When homologous Abs were used, it was possible to observe an increased expression of galectin-3 according to the dose of PMA used for treatment (Fig. 9, *left*). When an anti-TLR2 Ab was used for precipitation (Fig. 9, *right*), it was possible to reveal the presence of galectin-3 in the precipitates. This result depended on TLR2 expression by the cells. Absent in precipitates performed with untreated cells that did not express TLR2 (Fig. 9, *right*), galectin-3 was coprecipitated with TLR2, the amount of which depended on the dose of PMA used to differentiate the cells. Moreover, the lectin was not observed in precipitates in the absence of yeasts (data not shown).

Taken together with the previous results, these data strongly suggest that contact between the yeast cell wall and the cell membrane of differentiated macrophages specifically promotes the association of these two *C. albicans* interacting molecules.

Discussion

Specific interaction between galectins and their glycoconjugate ligands are considered to be critical determinants in pathogen recognition (28). Galectin has been proposed as a molecule involved in "seeing strangers or announcing danger" (29). First identified as an Ag expressed on the surface of murine thioglycolate-elicited macrophages, galectin-3 has been implicated in some aspects of innate immunity. Interestingly, the level of galectin-3 expression in myeloid cells may be related to cell activity toward invading microbes. Expression is very low in monocytes, but increases in mature macrophages (30) and immature dendritic cells (31), two types of cell dedicated to recognition of pathogens at the level of tissue penetration. However, its transcription is down-modulated in mature dendritic cells (31) when endophagocytosis and sensing are accomplished and the cells migrate to the lymph nodes.

Present at the plasma membrane, galectin-3 is also localized within the cytoplasm and nucleus (32). Presence of galectin-3 has been demonstrated at the level of phagocytic cups and phagosomes (33), where it seems to play an important role in the regulation of

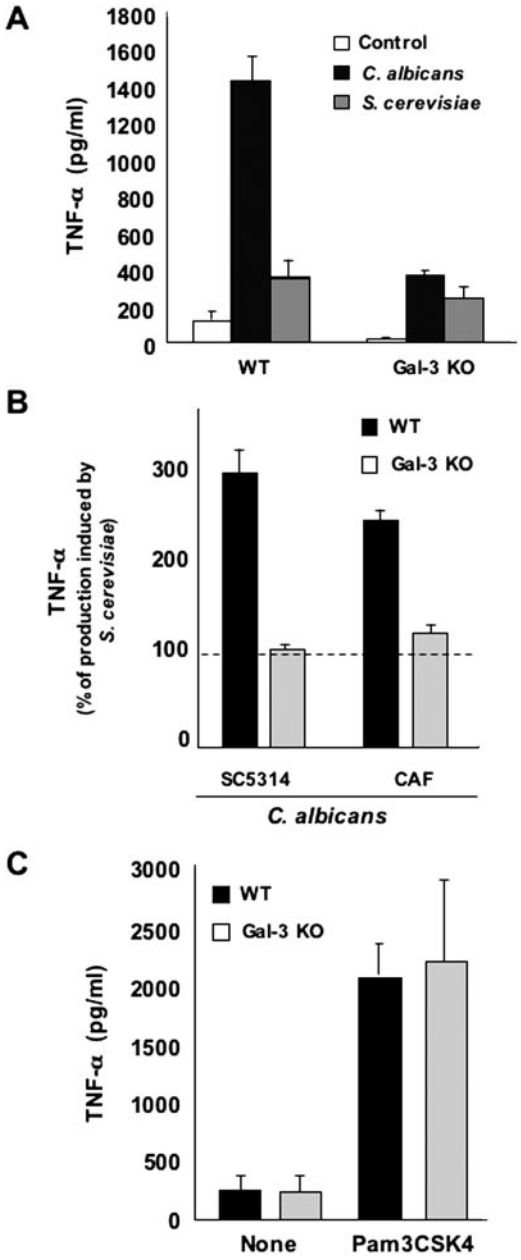

FIGURE 6. Comparison of TNF-α production by macrophages from WT or galectin-3 (Gal-3) KO mice in response to *C. albicans* or *S. cerevisiae*. *A*, Macrophages from WT or galectin-3 KO mice were incubated with either *C. albicans* or *S. cerevisiae* at a yeast to cell ratio of 5:1. TNF-α production was measured in cell-free supernatants after 3 h. Data are the mean of four independent experiments ± SD. *B*, Production of TNF-α induced by two different strains of *C. albicans* (SC5314 and CAF). Results are expressed as the percentage of production induced by *C. albicans*, 100% being the level of cytokine obtained with *S. cerevisiae*. *C*, Production of TNF-α by cells from WT or galectin-3 KO mice in response to 500 ng of TLR2 agonist Pam_3CSK_4. Data are the mean of three independent experiments ± SD.

phagolysosome maturation (34). An association with lysosomal membrane-associated glycoproteins has also been observed (35). Interestingly, although its physiological role remains unclear, galectin-3 promotes pathogen-macrophage interactions (36–42). For *C. albicans*, a recent proteomic study of infected cells showed that galectin-3 was one of the most regulated proteins whose expression was modulated during infection (43). However, although possible differential splicing has been observed leading to different

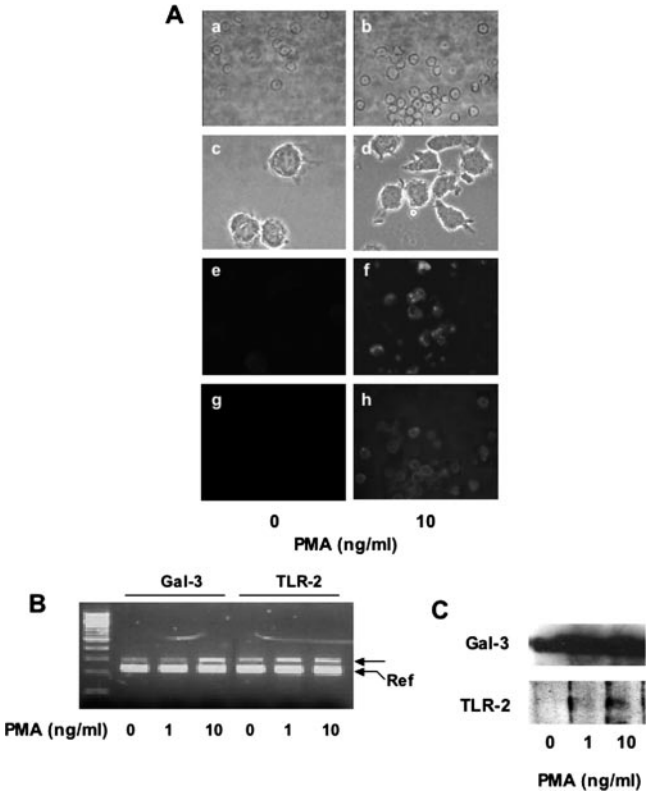

FIGURE 7. Differential expression of galectin-3 and TLR2 by THP-1 cells according to their degree of maturation induced by PMA. *A*, Maturation of THP-1 cells was followed after treatment without (*a*, *c*, *e*, and *g*) or with (*b*, *d*, *f*, and *h*) 10 ng/ml PMA. A change in cell adherence to culture wells (*a* and *b*) and morphologic evolution of the cells after treatment (*c* and *d*) are shown. Influence of PMA treatment on surface expression of galectin-3 (*e* and *f*) or TLR2 (*g* and *h*) by cells, as revealed by immunofluorescence using specific mAbs, is shown. *B*, Determination by RT-PCR of galectin-3- and TLR2-specific mRNA expression by cells after treatment with PMA. Mitochondrial ribosomal protein 28 S mRNA (Ref) is indicated. *C*, Revelation after immunoprecipitation with specific mAbs in Western blotting using homologous Abs to galectin-3 and TLR2 protein in THP-1 cells treated with different doses of PMA.

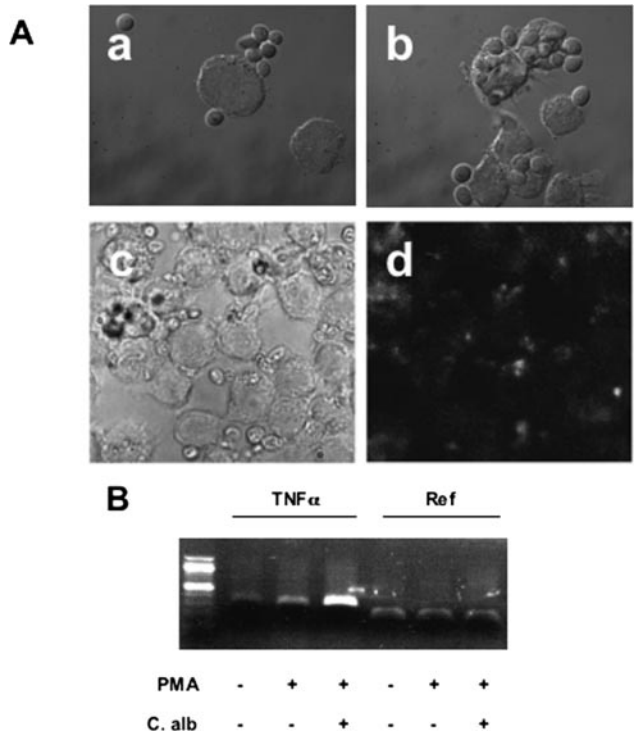

FIGURE 8. Effect of PMA treatment of THP-1 cells on endocytosis of *C. albicans*. *A*, Comparison of endocytic capability of cells untreated (*a*) or treated (*b*) with PMA toward *C. albicans* blastoconidia. Treated cells were incubated with *C. albicans* yeast cells and examined by phase-contrast microscopy. Localization of TLR2 in treated cells containing ingested *C. albicans* blastoconidia was performed after coculture by immunofluorescence with a specific mAb. Phase-contrast microcopy (*c*) and corresponding fluorescent staining (*d*) are shown. *B*, Determination by RT-PCR of TNF-α mRNA expression by cells incubated with *C. albicans* blastoconidia and treated with PMA is shown. GAPDH mRNA (Ref) is indicated.

proteins (44), the role of galectin-3 in signaling and recognition is still unclear because no transmembrane sequence or cytoplasmic tail has been demonstrated for this lectin (45).

Interaction of PAMPs with immune cells is now considered to be based on the assembly of a pluri-molecular complex that leads to signaling in the cells (2). This mechanism has been well documented for LPS, for which complex orchestration of multiple receptors, including TLR4 and CD14, has been demonstrated (46). Most of the pathogens interacting with galectin-3 also stimulate these cells through TLRs activation. This effect is the case for mycobacterial phosphatidylinositol mannosides, which have been shown to bind to galectin-3 (38) and to stimulate macrophages through TLR2 (47). By using either *C. albicans* or *S. cerevisiae*, we demonstrated that the interaction involving TLR2 and subsequent signaling leading to a proinflammatory response is not a trait specific to pathogens because both yeasts were able to stimulate cytokine production. However, although governed by identical signaling receptors (7), TNF-α induction was found to increase in the case of cells recognizing *C. albicans*. This finding suggests the involvement of another signal that would be associated with the specific recognition of this yeast. It has previously been shown that β-1,2 mannosides, which are specific for *C. albicans*, participate in the recognition process of this yeast by interacting with galectin-3 (23). In this study, the use of recombinant galectin-3 allowed us to demonstrate direct binding of the lectin at the surface of live yeasts where β-1,2 mannosides are expressed. This response contrasts with the reactivity of dectin-1 whose binding to live yeasts cells involving β-glucans is limited at some stages of growth and can only be increased by heating of the yeasts (11, 48, 49) or antifungal drugs (50). Interaction of *C. albicans* with galectin-3 was also observed directly onto the cells when the cellular distribution of the lectin was examined after endocytosis of *C. albicans*. In this case, as already described in other systems (33), galectin-3 could be localized at the level of the phagocytic cups formed around the yeasts and also at the periphery of ingested yeasts.

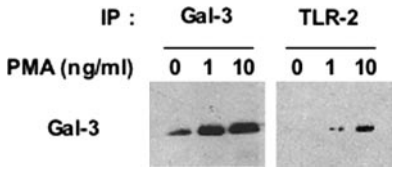

FIGURE 9. Galectin-3 and TLR2 coprecipitate in THP-1 cells after treatment with PMA and incubation with *C. albicans* blastoconidia. After treatment with different doses of PMA, THP-1 cells were incubated with *C. albicans* yeast cells. Proteins were extracted and incubated with either anti-galectin-3 or anti-TLR2 mAbs. Precipitates recovered on protein G-Sepharose beads were resolved by SDS-PAGE and transferred onto nitrocellulose. Presence of galectin-3 in the precipitates was then revealed by Western blotting with mAb to galectin-3.

Purified β-1,2 mannosides stimulated macrophages to produce TNF-α (20, 21). Such activity involving TLR2 (19) has also been shown for PLM, a glycolipid expressing β-1,2 mannosides (16) that binds to the macrophage membrane (18). The effect of deletion of galectin-3 on the cell response to yeasts was therefore investigated, hypothesizing that galectin-3 may associate with TLR2 for induction of specific stimulation by *C. albicans*. By using primary macrophages from galectin-3 KO mice, enhancement of the cell response toward *C. albicans*, which was observed with both macrophages from WT mice and the J774 cell line, was abrogated in cells that did not express galectin-3. In this case, *C. albicans* and *S. cerevisiae* blastoconidia induced similar cytokine production. This response could be explained by a lower capacity of the galectin-3-deficient cells to express TLRs or to recognize and/or endocytose the yeasts as suggested previously (34). However, comparison of cell response to specific agonist for TLRs revealed that both cell types expressed similarly TLR and that they presented identical capacity to produce cytokines. Moreover, endophagocytosis of both yeasts by macrophages from either WT or KO mice did not reveal any qualitative alteration at this step of the interaction. It therefore seems that two responses toward yeasts may exist. The first response, still stimulated in the absence of galectin-3, was induced by both yeast species; the second was induced specifically by *C. albicans* and depended on galectin-3 expression. This hypothesis, which agrees with results obtained in other systems (14), may explain for one part the observations obtained by Hobson et al. (51) who showed that disruption of the gene required for mannosyl phosphate transfer and, hence, the attachment of β-1,2 mannose oligosaccharides to mannan, did not alter the interaction between *C. albicans* and macrophages. An important drawback from the conclusions raised in this study is that, beside the fraction attached to mannan by mannosyl phosphate, a large amount of β-1,2 mannose oligosaccharides remained present at the cell wall surface, either attached to the mannan acid-stable part, the PLM, as well as to a large number of mannoproteins (52).

The level of galectin-3 has been shown to increase dramatically as monocytes differentiate into macrophages after culture in vitro or when cells were stimulated with phorbol esters (53). Among the different genes that are modulated in THP-1 cells in response to PMA, galectin-3 expression has been shown to be up-regulated by such treatment (30). We therefore took advantage of this model to explore the role of galectin-3 as a coreceptor for *C. albicans* recognition. Galectin-3 expression was revealed in cell extracts from undifferentiated cells and increased after PMA-induced differentiation. TLR2 protein was not observed in immature cells. Its expression increased according to the dose of PMA used for treatment. Interestingly, although no protein could be detected at the membrane or within cell extracts, a significant amount of the corresponding mRNA was apparent even in nontreated cells.

Although they expressed galectin-3, untreated THP-1 cells, which do not express TLR2, were not able to bind and endocytose *C. albicans* efficiently. Conversely, macrophages from galectin-3 KO mice phagocytosed the yeasts in a manner identical with macrophages from WT mice. After differentiation, THP-1 cells, which expressed both galectin-3 and TLR2, phagocytosed the yeasts. It therefore seems that galectin-3 is not critical for engulfment of yeasts and that endocytosis may be achieved only when the cells express TLR2. Although galectin-3 has been shown to be necessary for efficient IgG-mediated phagocytosis of erythrocytes by macrophages (34), these results show that the absence or presence of this lectin did not influence the binding and uptake of yeasts by the cells. In contrast, absence of TLR2 expression rendered the cells refractory to the yeasts, which confirms the results obtained by Blander and Medzhitov (54), who showed that phagocytosis of bacteria was impaired in the absence of TLR signaling. By using an anti-TLR2 Ab, it was possible to precipitate galectin-3 in extracts from cells that had been incubated with *C. albicans*. However, recovery of the lectin was dependent on the presence of TLR2 at the cell membrane because no galectin-3 was observed in precipitates obtained from undifferentiated cells that did not express TLR2 but nonetheless presented galectin-3. This observation suggests that galectin-3 may actually associate with TLR2 when both molecules are expressed at the membrane and after interaction with yeasts. Moreover, in the absence of TLR2 but presence of galectin-3 expression, which is the case of nondifferentiated THP-1 cells, no evidence of stimulation was obtained. In contrast, when both molecules were available, *C. albicans* induced a high level of TNF-α production. Previous results obtained with PLM (19, 55) or β-1,2 mannosides (our unpublished data) on undifferentiated monocytes have already suggested the need for cell maturation to gain optimal induction of cytokines.

Evidence of the existence of complex assembly has been established for yeasts interacting with macrophages. Proinflammatory activity of fungal β-glucans has been shown to depend on the cooperation between TLR2 and a dedicated coreceptor, dectin-1 (8), although recent findings showed that internalization of, and subsequent stimulation by, β-glucan particles depended mainly on dectin-1 expression rather than on TLR2 (56). For mannan, both TLR4 and CD14 have been shown to be necessary for signaling (57). Recently, an association of SIGNR1, a C-type lectin that binds α-linked mannosides equivalent to DC-SIGN that has been shown to interact with *C. albicans* (12), with the TLR4/MD2 complex has been shown to enhance signaling induced by LPS (14). Thus, although signaling seems to require an association with TLRs, coreceptors, and in particular lectins may cooperate for specific recognition of PAMPs. It is clear that all components of the yeast cell wall are differently recognized by specific interaction with lectin receptors. This recognition is the case for β-glucans, α-mannosides, and β-mannosides. The behavior of the yeasts and of the interacting cells should depend on the glycan and lectin involved in this recognition. The fact that for *C. albicans* it is possible to show an interaction that involves the association of a peculiar lectin and specific mannosides may explain why sensing of *C. albicans* by macrophages differs from other yeasts.

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Disclosures

The authors have no financial conflict of interest.

References

1. Takeda, K., and S. Akira. 2005. Toll-like receptors in innate immunity. *Int. Immunol.* 17: 1–14.
2. Taylor, P. R., L. Martinez-Pomares, M. Stacey, H.-H. Lin, G. D. Brown, and S. Gordon. 2005. Macrophage receptors and immune recognition. *Annu. Rev. Immunol.* 23: 901–944.
3. Mukhopadhyay, S., J. Herre, G. D. Brown, and S. Gordon. 2004. The potential for Toll-like receptors to collaborate with other innate immune receptors. *Immunology* 112: 521–530.
4. Calderone, R. A. 2002. *Candida and Candidiasis*. ASM Press, Washington, D.C.
5. Poulain, D., and T. Jouault. 2004. *Candida albicans* cell wall glycans, host receptors and responses: elements for a decisive crosstalk. *Curr. Opin. Microbiol.* 7: 342–349.
6. Roeder, A., C. J. Kirschning, R. A. Rupec, M. Schaller, G. Weindl, and H. C. Korting. 2004. Toll-like receptors as key mediators in innate antifungal immunity. *Med. Mycol.* 42: 485–498.
7. Netea, M. G., C. Van Der Graaf, J. W. Van Der Meer, and B. J. Kullberg. 2004. Recognition of fungal pathogens by Toll-like receptors. *Eur. J. Clin. Microbiol. Infect. Dis.* 23: 672–676.

8. Gantner, B. N., R. M. Simmons, S. J. Canavera, S. Akira, and D. M. Underhill. 2003. Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. *J. Exp. Med.* 197: 1107–1117.
9. Brown, G. D., J. Herre, D. L. Williams, J. A. Willment, A. S. Marshall, and S. Gordon. 2003. Dectin-1 mediates the biological effects of β-glucans. *J. Exp. Med.* 197: 1119–1124.
10. Brown, G. D., P. R. Taylor, D. M. Reid, J. A. Willment, D. L. Williams, L. Martinez-Pomares, S. Y. Wong, and S. Gordon. 2002. Dectin-1 is a major β-glucan receptor on macrophages. *J. Exp. Med.* 196: 407–412.
11. Gantner, B. N., R. M. Simmons, and D. M. Underhill. 2005. Dectin-1 mediates macrophage recognition of *Candida albicans* yeast but not filaments. *EMBO J.* 24: 1277–1286.
12. Cambi, A., K. Gijzen, J. M. de Vries, R. Torensma, B. Joosten, G. J. Adema, M. G. Netea, B.-J. Kullberg, L. Romani, and C. G. Figdor. 2003. The C-type lectin DC-SIGN (CD209) is an antigen-uptake receptor for *Candida albicans* on dendritic cells. *Eur. J. Immunol.* 33: 532–538.
13. Taylor, P. R., G. D. Brown, J. Herre, D. L. Williams, J. A. Willment, and S. Gordon. 2004. The role of SIGNR1 and the β-glucan receptor (Dectin-1) in the nonopsonic recognition of yeast by specific macrophages. *J. Immunol.* 172: 1157–1162.
14. Nagaoka, K., K. Takahara, K. Tanaka, H. Yoshida, R. M. Steinman, S. I. Saitoh, S. Akashi-Takamura, K. Miyake, Y. S. Kang, C. G. Park, and K. Inaba. 2005. Association of SIGNR1 with TLR4-MD-2 enhances signal transduction by recognition of LPS in Gram-negative bacteria. *Int. Immunol.* 17: 827–836.
15. Fradin, C., T. Jouault, A. Mallet, J. M. Mallet, D. Camus, P. Sinay, and D. Poulain. 1996. β-1,2-linked oligomannosides inhibit *Candida albicans* binding to murine macrophage. *J. Leukocyte Biol.* 60: 81–87.
16. Trinel, P. A., E. Maes, J. P. Zanetta, F. Delplace, B. Coddeville, T. Jouault, G. Strecker, and D. Poulain. 2002. *Candida albicans* phospholipomannan, a new member of the fungal mannose inositol phosphoceramide family. *J. Biol. Chem.* 277: 37260–37271.
17. Poulain, D., C. Slomianny, T. Jouault, J. M. Gomez, and P. A. Trinel. 2002. Contribution of phospholipomannan to the surface expression of β-1,2-oligomannosides in *Candida albicans* and its presence in cell wall extracts. *Infect. Immun.* 70: 4323–4328.
18. Jouault, T., C. Fradin, P. A. Trinel, A. Bernigaud, and D. Poulain. 1998. Early signal transduction induced by *Candida albicans* in macrophages through shedding of a glycolipid. *J. Infect. Dis.* 178: 792–802.
19. Jouault, T., S. Ibata-Ombetta, O. Takeuchi, P. Trinel, P. Sacchetti, P. Lefebvre, S. Akira, and D. Poulain. 2003. *Candida albicans* phospholipomannan is sensed through Toll-like receptors. *J. Infect. Dis.* 188: 165–172.
20. Jouault, T., G. Lepage, A. Bernigaud, P. A. Trinel, C. Fradin, J. M. Wieruszeski, G. Strecker, and D. Poulain. 1995. β-1,2-linked oligomannosides from *Candida albicans* act as signals for tumor necrosis factor α production. *Infect. Immun.* 63: 2378–2381.
21. Jouault, T., C. Fradin, P. A. Trinel, and D. Poulain. 2000. *Candida albicans*-derived β-1,2-linked mannooligosaccharides induce desensitization of macrophages. *Infect. Immun.* 68: 965–968.
22. Porcaro, I., M. Vidal, S. Jouvert, P. D. Stahl, and J. Giaimis. 2003. Mannose receptor contribution to *Candida albicans* phagocytosis by murine E-clone J774 macrophages. *J. Leukocyte Biol.* 74: 206–215.
23. Fradin, C., D. Poulain, and T. Jouault. 2000. β-1,2-linked oligomannosides from *Candida albicans* bind to a 32- kilodalton macrophage membrane protein homologous to the mammalian lectin galectin-3. *Infect. Immun.* 68: 4391–4398.
24. Trinel, P., C. Faille, P. Jacquinot, J. Cailliez, and D. Poulain. 1992. Mapping of *Candida albicans* oligomannosidic epitopes by using monoclonal antibodies. *Infect. Immun.* 60: 3845–3851.
25. Colnot, C., M. A. Ripoche, G. Milon, X. Montagutelli, P. R. Crocker, and F. Poirier. 1998. Maintenance of granulocyte numbers during acute peritonitis is defective in galectin-3-null mutant mice. *Immunology* 94: 290–296.
26. Jacquinot, P. M., Y. Plancke, B. Sendid, G. Strecker, and D. Poulain. 1998. Nature of *Candida albicans*-derived carbohydrate antigen recognized by a monoclonal antibody in patient sera and distribution over *Candida* species. *FEMS Microbiol. Lett.* 169: 131–138.
27. Káposzta, R., L. Marodi, M. Hollinshead, S. Gordon, and R. da Silva. 1999. Rapid recruitment of late endosomes and lysosomes in mouse macrophages ingesting *Candida albicans*. *J. Cell Sci.* 112: 3237–3248.
28. Rabinovich, G. A., and A. Gruppi. 2005. Galectins as immunoregulators during infectious processes: from microbial invasion to the resolution of the disease. *Parasite Immunol.* 27: 103–114.
29. Sato, S., and J. Nieminen. 2004. Seeing strangers or announcing "danger": galectin-3 in two models of innate immunity. *Glycoconj J.* 19: 583–591.
30. Kim, K., E. P. Mayer, and M. Nachtigal. 2003. Galectin-3 expression in macrophages is signaled by Ras/MAP kinase pathway and up-regulated by modified lipoproteins. *Biochim. Biophys. Acta* 1641: 13–23.
31. Dietz, A. B., P. A. Bulur, G. J. Knutson, R. Matasić, and S. Vuk-Pavlović. 2000. Maturation of human monocyte-derived dendritic cells studied by microarray hybridization. *Biochem. Biophys. Res. Commun.* 275: 731–738.
32. Patterson, R., W. Wang, and J. Wang. 2004. Understanding the biochemical activities of galectin-1 and galectin-3 in the nucleus. *Glycoconj J.* 19: 499–506.
33. Garin, J., R. Diez, S. Kieffer, J.-F. Dermine, S. Duclos, E. Gagnon, R. Sadoul, C. Rondeau, and M. Desjardins. 2001. The phagosome proteome: insight into phagosome functions. *J. Cell Biol.* 152: 165–180.
34. Sano, H., D. K. Hsu, J. R. Apgar, L. Yu, B. B. Sharma, I. Kuwabara, S. Izui, and F. T. Liu. 2003. Critical role of galectin-3 in phagocytosis by macrophages. *J. Clin. Invest.* 112: 389–397.
35. Sarafian, V., M. Jadot, J.-M. Foidart, J.-J. Letesson, F. Van den Brûle, V. Castronovo, R. Wattiaux, and S. W. Coninck. 1998. Expression of Lamp-1 and Lamp-2 and their interactions with galectin-3 in human tumor cells. *Int. J. Cancer* 75: 105–111.
36. Gupta, S. K., S. Masinick, M. Garrett, and L. D. Hazlett. 1997. *Pseudomonas aeruginosa* lipopolysaccharide binds galectin-3 and other human corneal epithelial proteins. *Infect. Immun.* 65: 2747–2753.
37. Moody, T. N., J. Ochieng, and F. Villalta. 2000. Novel mechanism that *Trypanosoma cruzi* uses to adhere to the extracellular matrix mediated by human galectin-3. *FEBS Lett.* 470: 305–308.
38. Beatty, W. L., E. R. Rhoades, D. K. Hsu, F.-T. Liu, and D. G. Russell. 2002. Association of a macrophage galactoside-binding protein with *Mycobacterium*-containing phagosomes. *Cell. Microbiol.* 4: 167–176.
39. John, C. M., G. A. Jarvis, K. V. Swanson, H. Leffler, M. D. Cooper, M. E. Huflejt, and J. M. Griffiss. 2002. Galectin-3 binds lactosaminylated lipooligosaccharides from *Neisseria gonorrhoeae* and is selectively expressed by mucosal epithelial cells that are infected. *Cell. Microbiol.* 4: 649–662.
40. Pelletier, I., and S. Sato. 2002. Specific recognition and cleavage of galectin-3 by *Leishmania major* through species-specific polygalactose epitope. *J. Biol. Chem.* 277: 17663–17670.
41. van den Berg, T. K., H. Honing, N. Franke, A. van Remoortere, W. E. Schiphorst, F.-T. Liu, A. M. Deelder, R. D. Cummings, C. H. Hokke, and I. van Die. 2004. LacdiNAc-glycans constitute a parasite pattern for galectin-3-mediated immune recognition. *J. Immunol.* 173: 1902–1907.
42. Vray, B., I. Camby, V. Vercruysse, T. Mijatovic, N. V. Bovin, P. Ricciardi-Castagnoli, H. Kaltner, I. Salmon, H.-J. Gabius, and R. Kiss. 2004. Up-regulation of galectin-3 and its ligands by *Trypanosoma cruzi* infection with modulation of adhesion and migration of murine dendritic cells. *Glycobiology* 14: 647–657.
43. Shin, Y. K., H. J. Lee, J. S. Lee, and Y. K. Paik. 2006. Proteomic analysis of mammalian basic proteins by liquid-based two-dimensional column chromatography. *Proteomics* 6: 1143–1150.
44. Gorski, J. P., F. T. Liu, A. Artigues, L. F. Castagna, and P. Osdoby. 2002. New alternatively spliced form of galectin-3, a member of the β-galactoside-binding animal lectin family, contains a predicted transmembrane-spanning domain and a leucine zipper motif. *J. Biol. Chem.* 277: 18840–18848.
45. Liu, F. T. 2005. Regulatory roles of galectins in the immune response. *Int. Arch. Allergy Immunol.* 136: 385–400.
46. Triantafilou, M., and K. Triantafilou. 2005. The dynamics of LPS recognition: complex orchestration of multiple receptors. *J. Endotoxin Res.* 11: 5–11.
47. Jones, B. W., K. A. Heldwein, T. K. Means, J. J. Saukkonen, and M. J. Fenton. 2001. Differential roles of Toll-like receptors in the elicitation of proinflammatory responses by macrophages. *Ann. Rheum. Dis.* 60(Suppl. 3): iii6–iii12.
48. Heinsbroek, S. E., G. D. Brown, and S. Gordon. 2005. Dectin-1 escape by fungal dimorphism. *Trends Immunol.* 26: 352–354.
49. Gersuk, G. M., D. M. Underhill, L. Zhu, and K. A. Marr. 2006. Dectin-1 and TLRs permit macrophages to distinguish between different *Aspergillus fumigatus* cellular states. *J. Immunol.* 176: 3717–3724.
50. Wheeler, R. T., and G. R. Fink. 2006. A drug-sensitive genetic network masks fungi from the immune system. *PloS Pathog.* 2: e35.
51. Hobson, R. P., C. A. Munro, S. Bates, D. M. MacCallum, J. E. Cutler, S. E. Heinsbroek, G. D. Brown, F. C. Odds, and N. A. Gow. 2004. Loss of cell wall mannosylphosphate in *Candida albicans* does not influence macrophage recognition. *J. Biol. Chem.* 279: 39628–39635.
52. Mille, C., G. Janbon, F. Delplace, S. Ibata-Ombetta, C. Gaillardin, G. Strecker, T. Jouault, P. A. Trinel, and D. Poulain. 2004. Inactivation of CaMIT1 inhibits *Candida albicans* phospholipomannan β-mannosylation, reduces virulence and alters cell wall protein β-mannosylation. *J. Biol. Chem.* 279: 47952–47960.
53. Nangia-Makker, P., J. Ochieng, J. K. Christman, and A. Raz. 1993. Regulation of the expression of galactoside-binding lectin during human monocytic differentiation. *Cancer Res.* 53: 5033–5037.
54. Blander, J. M., and R. Medzhitov. 2004. Regulation of phagosome maturation by signals from Toll-like receptors. *Science* 304: 1014–1018.
55. Jouault, T., A. Bernigaud, G. Lepage, P. A. Trinel, and D. Poulain. 1994. The *Candida albicans* phospholipomannan induces in vitro production of tumour necrosis factor-α from human and murine macrophages. *Immunology* 83: 268–273.
56. McCann, F., E. Carmona, V. Puri, R. E. Pagano, and A. H. Limper. 2005. Macrophage internalization of fungal β-glucans is not necessary for initiation of related inflammatory responses. *Infect. Immun.* 73: 6340–6349.
57. Tada, H., E. Nemoto, H. Shimauchi, T. Watanabe, T. Mikami, T. Matsumoto, N. Ohno, H. Tamura, K. Shibata, S. Akashi, et al. 2002. *Saccharomyces cerevisiae*- and *Candida albicans*-derived mannan induced production of tumor necrosis factor α by human monocytes in a CD14- and Toll-like receptor 4-dependent manner. *Microbiol. Immunol.* 46: 503–512.