MAJOR ARTICLE

Candida albicans Phospholipomannan Is Sensed through Toll-Like Receptors

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Candida albicans **is a common, harmless yeast in the human digestive tract that also causes severe systemic fungal infection in hospitalized patients. Its cell-wall surface displays a unique glycolipid called phospholipomannan (PLM). The ability of PLM to stimulate tumor necrosis factor (TNF)–α production by J774 mouse cells correlates with the activation of nuclear factor (NF)–κB. We examined the involvement of Toll-like receptors (TLRs) in PLM-dependent stimulation. Compared with wild-type cells, which produced large amounts of TNF-α after incubation with PLM, the deletion of the TLR4 and TLR6 genes led to a limited alteration of the PLM-induced response. Deletion of the TLR2 gene completely abolished the cell response. Surface expression of PLM is a phylogenic trait of *C. albicans,* and the recognition of PLM by TLRs, together with the unique pathogenic potential of *C. albicans,* suggests that this molecule may be a member of the pathogen-associated molecular pattern family.**

The increasing incidence of systemic fungal infections in hospitalized patients has been a constant feature of infectious diseases over recent decades [1]. Among these, infections caused by *Candida* species rank fourth among nosocomial infections [1]. *Candida albicans* is the most frequently isolated pathogen [2] when the delicate balance between the host and this otherwise harmless commensal can turn into a parasitic relationship, resulting in the development of severe infections. *C. albicans* is a significant cause of morbidity and mortality in hospitals, where the manifestation and severity of infection depend on the nature and extent of the impairment to the host's immune defense.

Clinical evidence and experimental data have indicated that both the innate and adaptive immune systems regulate the control of and resistance to *Candida* infections [3]. The fungus is not a mere passive participant in the infectious process. The specific abilities of *C. albicans* to alter its phenotype and cell shape by producing filaments, to express specific adhesins, and to secrete enzymes like secreted aspartyl proteinases and phospholipases have been well characterized [1]. Modulation of the host's immune response by *C. albicans* is another important factor that influences the development of infection [3]. *C. albicans* or its constituents have been shown to directly modulate the response of accessory cells of innate immunity [4–8]. For example, *C. albicans* induces the apoptosis of neutrophils [9] and interferes with the transduction of signals necessary for the lytic activity of macrophages [10].

In a series of articles, it has been shown that *C. albicans* expresses a glycolipid, phospholipomannan (PLM) [11–13], that has potent activity on the innate immune response, which leads to the production of proinflammatory mediators by cells of myeloid lineage [14, 15]. An analysis of the structure of *C. albicans* PLM has been completed recently and showed that PLM is composed of hydroxy fatty acid amide linked to phytosphyngosine, with a hydrophilic polysaccharide domain that consists

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of a linear chain of β-1,2–linked mannose residues [16]. PLM is shed by yeasts on contact with macrophages and binds to these cells, inducing tumor necrosis factor (TNF)–α secretion and protein tyrosine kinase–dependent signal transduction, similar to that induced by whole yeast cells [15].

Toll-like receptors (TLRs), a family of proteins that are homologous to *Drosophila* toll protein (Toll), have been shown to be critical in sensing invading surface molecules from microorganisms [17] that initiate a signaling cascade, resulting in proinflammatory cytokine production [18, 19]. Antimicrobial defenses involving macrophages are based mainly on recognition by TLR2, TLR4, and TLR6, depending on the nature of the ligands [20]. It is now agreed that TLR4 is the signal-transducing receptor for lipopolysaccharide (LPS) from gram-negative bacteria [21, 22]. TLR2 interacts with bacterial lipoproteins [23] and whole gram-positive bacteria [24], is recruited by macrophage phagosomes, and discriminates between yeasts and other pathogens [25]. Recently, TLR2 expression has been shown to be necessary for *C. albicans*–induced TNF-α secretion by macrophages, whereas the absence of TLR4 impaired chemokine secretion and subsequent neutrophil recruitment [26].

The *C. albicans* cell wall is a complex structure composed of chitin, glucans, mannoproteins, mannans, and glycolipids. Some of these molecular entities are similar in *C. albicans* and nonpathogenic yeasts of the same phylum. However, until now, experiments have used heat-killed yeasts, and the nature of the molecules presented to the host cells had not been determined. PLM shares most of the characteristics of the so-called pathogen-associated molecular patterns (PAMPs) [14–16, 27], which are microbial components recognized by TLRs. It was therefore tempting to speculate that PLM, like other microbial glycolipids, also uses TLRs for the activation of the immune cells. In the present study, macrophages were stimulated to produce the proinflammatory cytokine TNF-α in a TLR-dependent manner on exposure to *C. albicans* PLM. TLR2 was the major mediator of the proinflammatory signal induced by PLM.

MATERIALS AND METHODS

Reagents and antibodies. All reagents were obtained from Sigma-Aldrich Chimie, unless otherwise stated. Mouse monoclonal antibody (MAb) IgG specific for the p65/RelA subunit of NF-κB (clone F-6) and rabbit polyclonal IgG to TLR2 (clone H-175) were obtained from Santa Cruz Biotechnology. Horseradish peroxidase (HRPO)– and fluorescein isothiocyanate (FITC)–conjugated anti–mouse IgG and anti–rabbit IgG were obtained from Southern Biotechnology Laboratories.

Yeast culture and PLM purification. *C. albicans* VW32 (serotype A) was used throughout the study. Yeasts were maintained on Sabouraud's dextrose agar at 4°C. *C. albicans* PLM was prepared by extensive purification partition and hydrophobic interaction steps, as described elsewhere [16]. The structure of this molecule was determined by a combination of methanolysis/high-performance liquid chromatography, phosphorus/proton nuclear magnetic resonance, and matrix-associated laser desorption/ionization–time-of-flight mass-spectrometry methods. The present study used the *C. albicans* PLM batch recovered from those structural studies [16], after analysis by nondenaturing methods.

J774 cell line and mouse peritoneal macrophages. The mouse macrophage-like cell line J774 (ECACC 85011428) was derived from a tumor from a female BALB/c mouse. Adherent cells were cultured at 37°C in an atmosphere that contained 5% CO_2 in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum (FCS; Valbiotech), 5 mmol L-glutamine, 100 μg/mL streptomycin and 50 μg/mL penicillin. Before use, cells were gently scraped off with a rubber policeman and, depending on the experiment, either plated into 8-well Labtek tissue-culture chambers (Nunc) at a concentration of 0.5×10^6 cells/well for immunofluorescence assays or into 24- or 48-well tissue culture dishes at a concentration of 10^6 cells/well in 500 μL of culture medium (for biochemical analysis or detection of TNF-α production in cell-free supernatants, respectively).

Wild-type, TLR2 knockout (KO), TLR4 KO, and TLR6 KO mice [21] were inoculated intraperitoneally with 2 mL of 4% thioglycolate (Difco Laboratories); 3 days later, peritoneal exudate cells were harvested in cold serum-free RPMI 1640 medium. Peritoneal macrophages (2×10^5 cells/well) were dispensed into a 96-well plate in RPMI 1640 medium supplemented with 10% FCS. After washing, adherent cells were incubated with 50 μg/mL PLM in the presence of 30 U/mL interferon (IFN)–γ for 24 h.

Immunofluorescence analysis. After stimulation for 60 min at 37°C with PLM (10 and 50 μg/mL) or LPS (10 μg/mL, used as a control) in culture medium, J774 cells were washed with warm DMEM and fixed and permeabilized with 3.7% formaldehyde and 0.2% Triton X-100 in phosphate buffer at 20°C for 20 min. After 3 washes with phosphate buffer, 100 μL of a 1:100 dilution of either rabbit polyclonal IgG to TLR2 or anti–NF-κB p65/RelA mouse MAb IgG was added for 2 h at 20°C. After 5 washes, 100 μL of a 1:100 dilution of FITC-conjugated goat anti–rabbit or anti–mouse IgG in phosphate buffer was added for 1 h at 20°C. Slides were then washed 5 times and mounted for microscopic examination.

Nuclear extract preparation and Western blot analysis of P65/RelA translocation. Nuclear protein extracts were prepared from 10^6 J774 cells stimulated for 90 min with 10 or 50 μg/mL PLM or 10 μg/mL LPS. Cells were washed in 1.0 mL of ice-cold PBS, and the cell pellets were lysed in 400 μL of lysis buffer (10 mmol HEPES, 10 mmol KCl, 0.1 mmol EDTA, 0.1 mmol EGTA, 0.5% Nonidet P-40, 1 mmol dithiothreitol [DTT], and 0.5 mmol

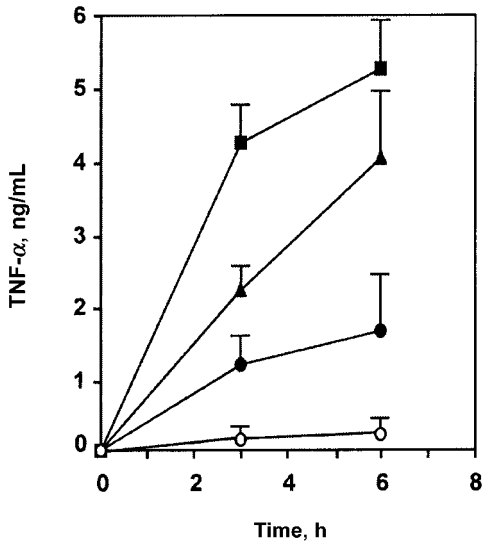

Figure 1. Phospholipomannan (PLM) purified from *Candida albicans* stimulates tumor necrosis factor (TNF)–α production by J774 cells. J774 macrophages were incubated at 37°C without *(white circles)* or with *(black circles)* 5, *(black triangles)* 10, or *(black squares)* 50 μg/mL PLM. Supernatants were collected at different times. TNF-α bioactivity was measured in a lytic assay that used the L929 cells. Results are expressed as the mean ± SD of triplicate determinations from 1 of 3 similar experiments.

phenylmethylsulfonyl fluoride [PMSF]) in the presence of protease inhibitors (antipain, aprotinin, leupeptin, bestatin, phosphoramidon, pepstatin, and soybean trypsin inhibitor, all at a concentration of 1 μg/mL). Cells were incubated on ice for 15 min. The cell lysates were subsequently centrifuged for 10 s at 1000 *g*. The nuclear pellet from lysis was resuspended in 50 μL of nuclear buffer (0.4 mol NaCl, 20 mmol HEPES [pH 7.9], 25% glycerol, 1 mmol EDTA, 1 mmol EGTA, 1 mmol DTT, and 1 mmol PMSF, in the presence of protease inhibitors) at 4°C with extensive shaking for 30 min. Cell debris was removed by centrifugation for 10 min at 10,000 *g*, and fractions were stored at −70°C until analysis. Protein concentrations were determined by spectrophotometric assay (Bio-Rad), according to the manufacturer's specifications.

Nuclear proteins (60 μg) were separated by 10% SDS-PAGE, transferred to nitrocellulose membranes, and blocked with 5% nonfat milk in Tris-buffered saline/Tween. Proteins were subsequently immunoblotted with anti–NF-κB p65/RelA mouse MAb IgG. Immunoreactive proteins were detected with HRPO-conjugated anti–mouse IgG using an enhanced chemiluminescence assay.

Electrophoretic mobility shift assay. Double-stranded oligonucleotides containing a NF-κB consensus (5′-AGT TGA GGG GAC TTT CCC AGG C-3′) (Santa Cruz) were used as probes. DNA probes were labeled with ^{32}P-deoxynucleotide triphosphate using T4 polynucleotide kinase. Nuclear extracts (1, 3, and 6 μg of proteins) were incubated at room temperature for 20 min with 0.5 ng of radiolabeled-DNA probe (30,000 cpm) in binding buffer (10 mmol Tris HCl [pH 8.0], 40 mmol KCl, 18% glycerol, 0.05% Nonidet P-40, 1 mmol DTT, 2 μL of 0.5 mol benzamidine, 5 μL of 0.1 mol PMSF, and 1 μg of poly[dI-dC]). DNA-protein complexes were resolved by electrophoresis through 5.5% polyacrylamide gels. The specificity of the interaction between nuclear proteins and the NF-κB binding site was determined by the addition of 20-fold excess cold oligonucleotide to the DNA-protein complexes before electrophoresis. The gels were dried, exposed to a phosphoimager screen, and analyzed using Imagequant software (Molecular Dynamics).

TNF-α production by stimulated cells. J774 cells were incubated with medium alone or with different concentrations (5, 10, and 50 μg/mL) of PLM. After different periods of time, 50 μL of cell-free supernatant was collected and stored at −80°C. The TNF-α concentration was measured by a lytic assay that used the TNF-sensitive mouse fibroblast cell line, L929, as described elsewhere [15].

For peritoneal macrophages from KO mice, the production of TNF-α was measured after 24 h of stimulation in the presence or absence of 50 μg/mL PLM, together with 30 U/mL IFN-γ. The concentration of the cytokine in cell-free supernatants was determined by ELISA (Genzyme Techne).

RESULTS

PLM induces TNF-α production in J774 cells. We have shown in previous studies that *C. albicans* PLM stimulated TNF-α production by macrophages [14, 15]. To further explore the mechanism of PLM-induced activation, we used highly purified PLM batches whose structure has been fully characterized recently [16] and murine macrophage J774 cells, which have been shown to respond to PLM [15] and express TLRs [28, 29]. The capability of PLM to induce TNF-α production by these cells was first examined in a bioassay. As shown in figure 1, significant TNF-α production was seen after incubation with 10 μg/mL PLM. The induction of cytokine production depended on the dose of PLM added to the cells; the maximum effect was obtained when cells were incubated with 50 μg/mL PLM.

PLM activates the nuclear translocation of the p65/RelA subunit of NF-κB in J774 macrophages. In macrophages, transcriptional activation and the subsequent release of TNF-α require the activation of NF-κB [30]. We therefore examined the involvement of NF-κB in the PLM-dependent activation of J774 cells. As shown in figure 2, the incubation of J774 cells with PLM or with high doses of LPS used as a positive control for induction of NF-κB activation [31] resulted in the translocation of the p65/RelA subunit of NF-κB from the cytosol to

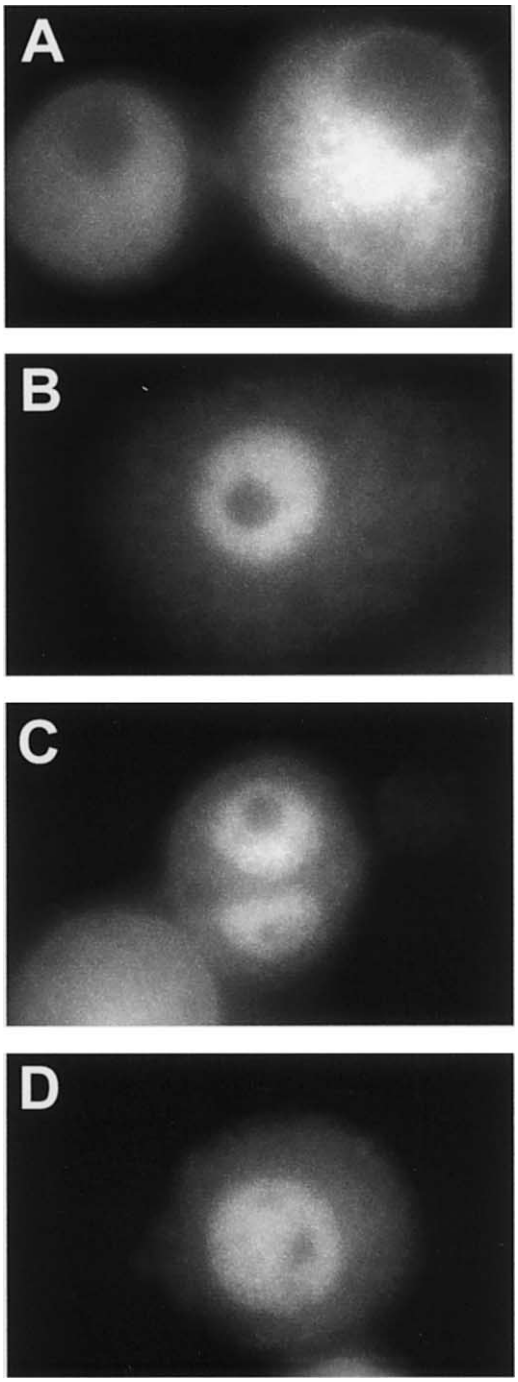

Figure 2. *Candida albicans* phospholipomannan (PLM) induces p65/RelA translocation in J774 cells. J774 macrophages were incubated in the presence of medium alone *(A),* 10 μg/mL lipopolysaccharide *(B),* or 10 *(C)* or 50 *(D)* μg/mL PLM. After 60 min, the cells were washed 3 times with phosphate buffer. After fixation and permeabilization with 3.7% paraformaldehyde and 0.2% Triton X-100 in PBS for 15 min at 20°C, cells were incubated for 2 h at 20°C with 100 μL of a 1:100 dilution of anti–NF-κB p65/RelA mouse monoclonal antibody (MAb) IgG. Bound MAb was revealed with fluorescein isothiocyanate–conjugated goat anti–mouse IgG.

the nucleus. In the absence of any stimulus, the p65/RelA subunit was localized exclusively within the cytosol compartment of J774 cells even after 60 min of incubation (figure 2*A*). Stimulation of cells for 30 min with LPS led to a transient localization of p65/RelA both within the cytosol and in the nucleus (data not shown), and, after 60 min of incubation, p65/RelA localized mainly within the nucleus of most cells (figure 2*B*). At this time, the translocation of p65/RelA depended on the PLM concentration used for the stimulation of the cells: with 10 μg/mL, p65/RelA was localized in both the cytosol and the nucleus of cells (figure 2*C*); with 50 μg/mL PLM, most p65/RelA was localized within the nucleus of the cells (figure 2*D*).

To confirm that p65/RelA translocated within the cell nucleus after stimulation with PLM, Western blot analysis was done on nuclear extracts of cells stimulated for 90 min with 50 μg/mL PLM (figure 3). Compared with control cells incubated without stimulus (figure 3, *lane 1*), the level of p65/RelA clearly increased in nuclear extracts from cells stimulated with PLM (figure 3, *lane 2*) and with LPS used as a control (figure 3, *lane 3*).

The interaction of p65/RelA with the NF-κB DNA consensus site was subsequently demonstrated by gel-shift analysis. J774 cells were incubated without or with PLM, and different concentrations of proteins extracted from cell nuclei were assayed for NF-κB DNA-binding activities, using a radiolabeled NF-κB–specific probe. Compared with unstimulated cells (figure 4*A, lanes 1-3*), nuclear extracts from PLM-stimulated cells formed complexes binding to the NF-κB DNA binding consensus site (figure 4*A, lanes 4–6*) that were dependent on the concentration of nuclear protein used. To examine the specificity of the DNA-binding capability of the complexes generated by PLM, a 20-fold excess of unlabeled NF-κB oligonucleotides was added for competition (figure 4*B*). As we had expected, the unlabeled oligonucleotides prevented the formation of radiolabeled protein-DNA complexes (figure 4*B, lane 2*). Together, these results indicate that both TNF-α production and NF-κB translocation within the nucleus are induced in J774 cells after stimulation by PLM.

Involvement of TLRs in PLM-induced cytokine production. The induction of NF-κB activation is recognized as a key for signals initiated at the macrophage membrane by TLRs. We, therefore, investigated the role of TLRs in the recognition of PLM by macrophages. Peritoneal macrophages isolated from either wild-type mice or 3 KO mice (TLR2$^{-/-}$, TLR4$^{-/-}$, and TLR6$^{-/-}$) [21] were incubated with PLM, and the subsequent TNF-α production in cell-free supernatants was measured. The effect of PLM incubation on TNF-α secretion by macrophages from wild-type mice was first verified. Compared with unstimulated cells, significant TNF-α production was observed after 24 h of incubation with 50 μg/mL PLM (figure 5). However, as had already been seen with primary macrophages [14], the presence of IFN-γ was necessary to obtain the optimal induc-

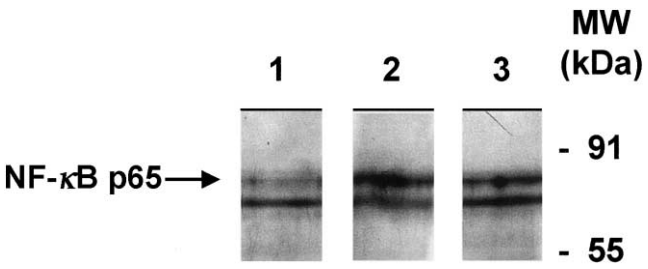

Figure 3. Nuclear extracts from phospholipomannan (PLM)–stimulated cells contained p65/RelA. J774 macrophages were incubated for 90 min in the presence of medium alone *(lane 1)* or in the presence of 50 *(lane 2)* μg/mL PLM or 10 *(lane 3)* μg/mL lipopolysaccharide. Sixty-microgram proteins from nuclear extracts were loaded in each lane and separated by 10% SDS-PAGE before transfer to nitrocellulose. Membranes were probed with anti–NF-κB p65/RelA mouse monoclonal antibody IgG. MW, molecular weight.

tion of cytokine production with these cells. These conditions were therefore used for stimulation of cells isolated from KO mice. The results are summarized in figure 5. Compared with the TNF-α production observed with cells from wild-type mice (4.23 ± 0.24 ng/mL), the absence of TLR4 expression by these cells resulted in a 53% inhibition of TNF-α production (1.97 ± 0.09 ng/mL) (figure 5*A*). The results obtained with macrophages from TLR6 KO mice were quite similar (figure 5*B*). TNF-α production by these cells after incubation with 50 μg/mL PLM was decreased, resulting in a 61% inhibition of TNF-α production, compared with cells isolated from wild-type mice. In contrast, deletion of the TLR2 gene dramatically altered the capability of cells to respond to PLM (figure 5*A*). TNF-α production was reduced from 4.23 ± 0.24 to 0.26 ± 0.01 ng/mL for cells from wild-type and TLR2 KO mice, respectively, which corresponded to a >90% inhibition of cytokine production in response to PLM stimulation.

DISCUSSION

C. albicans has been shown to stimulate cells of the macrophage lineage to produce proinflammatory cytokines. The interaction of yeasts with macrophages is mediated by the cell wall [32], a structure that is rich in polysaccharide components. Among these, some have their counterpart host-specific receptors, such as the macrophage mannose receptor for α-mannosides [33], the β-glucan receptor for β-1,6 glucans [34], and the recently described receptor dectin-1 of β-1,3 glucans [35]. It has been shown recently that binding a special type of mannose residue present in *C. albicans* mannan, β-1,2–oligomannoside [36], to galectin-3 provides an alternative receptor for *C. albicans* [37]. β-1,2–oligomannosides are also present in *C. albicans*, in the glycan moiety of the glycolipid PLM [16]. Although the stimulatory activity of PLM toward macrophages has been demonstrated [14, 15], the mechanism(s) of the recognition of PLM by these cells has not been investigated.

The activation of NF-κB after stimulation by PLM was highlighted by translocation of the p65/RelA subunit from the cytosol to the nucleus of activated cells and, ultimately, binding to the DNA consensus site. Although different upstream pathways have been described in TLR-induced NF-κB activation, this central step in transduction represents one of the features of TLR-dependent signaling in macrophages [38]. We therefore proposed the possible involvement of these receptors in initiating the cell response to PLM.

Glycoconjugate-dependent cell stimulation is initiated mainly by TLR2 or TLR4, depending on the nature of the stimulus [20]. J774 cells used have been shown to express both TLRs [28, 29]. Immunofluorescence studies on our J774 cell line used as target cells for PLM have confirmed the expression of TLR2 localized at the plasma membrane and in vesicles, as has been reported elsewhere with monocyte-derived macrophages [39] (data not shown). To explore the role of these receptors in PLM-induced

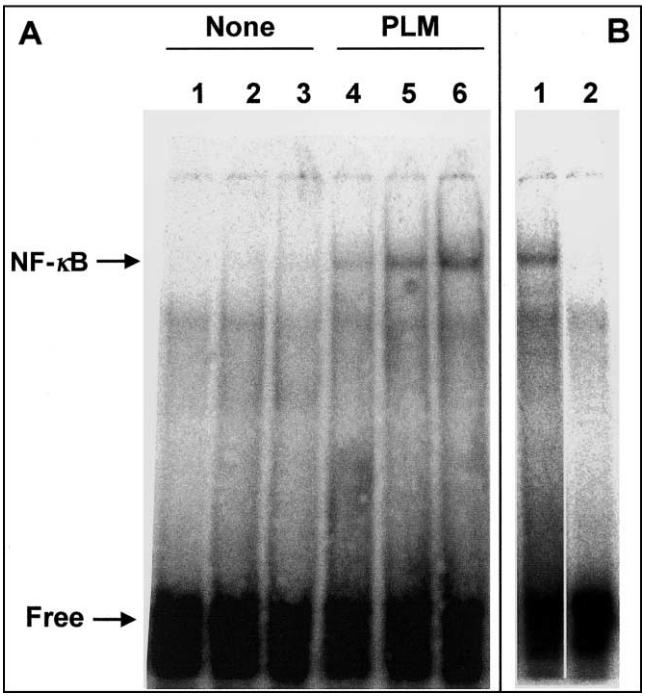

Figure 4. *Candida albicans* phospholipomannan (PLM) activates NF-κB in J774 cells. NF-κB activity in nuclear extracts from J774 cells was examined by gel-shift assays. *A,* The NF-κB probe was incubated with 1 *(lanes 1* and *4),* 3 *(lanes 2* and *5),* and 6 *(lanes 3* and *6)* μg of protein from nuclear extracts of cells incubated for 90 min without *(lanes 1–3)* or with 10 μg/mL *(lanes 4–6)* PLM. *B,* The specificity of the DNA-binding capacity of the complexes generated after incubation with PLM is shown. Before electrophoresis, a 20-fold excess of unlabeled oligonucleotides was added *(lane 2)* or not *(lane 1)* to complexes obtained with 6 μg of protein from PLM-stimulated cells. The results shown are representative of 3 independent experiments.

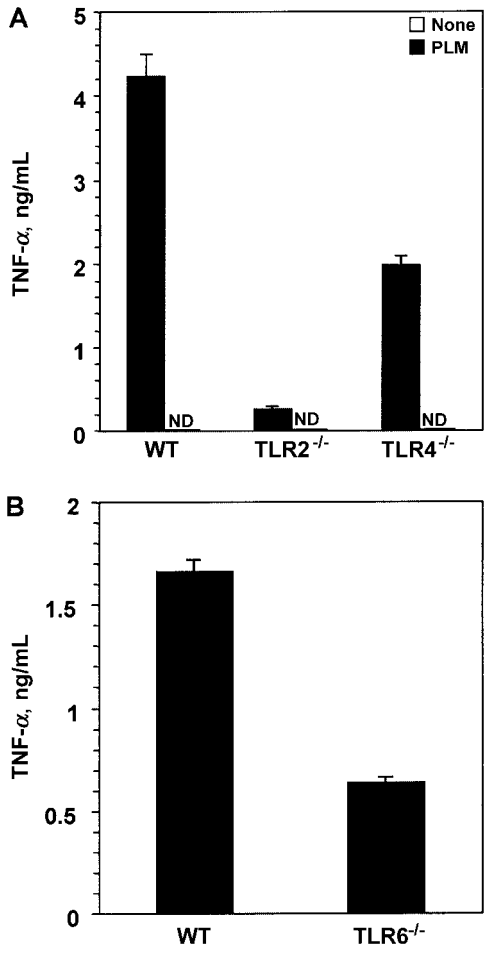

Figure 5. The involvement of Toll-like receptor (TLR) 2, TLR4, and TLR6 in the activation of murine macrophages by phospholipomannan (PLM) is shown. Peritoneal macrophages were isolated from wild-type *(wt)* and TLR knockout (KO) mice. After 24 h of incubation with 50 μg/mL PLM, supernatants were collected, and tumor necrosis factor (TNF)–α production was measured by ELISA. The results, which are expressed as the mean $\pm$ SD of triplicate determinations of 1 representative experiment, show TNF-α production by macrophages from *wt* mice, compared with macrophages isolated from TLR2 or TLR4 KO mice *(A)* and TLR6 KO mice *(B)*. ND, not detected.

TNF-α production and to assess their discriminating role in PLM recognition, primary macrophages isolated from different TLR KO mice were used. The results obtained with these cells clearly showed that TLR2 expression was important for stimulation by PLM—the absence of this receptor led to the almost complete unresponsiveness of the cells to PLM. However, cells expressing TLR2, but not TLR4 or TLR6, also showed decreased TNF-α production after incubation with PLM but to a lesser extent.

Several articles have demonstrated the involvement of TLR2 in the recognition of glycolipids from a large number of phylogenetically unrelated pathogens, such as lipoarabinomannan from *Mycobacteria* species [40], lipoteichoic acid from *Staphylococcus* species, glycolipids from *Treponema* species [41], and glycosylinositolphospholipid from *Trypanosoma* species [42]. However, the complex formation of TLR2 with other molecules involved in pattern recognition—such as CD14, MD2, TLR1, TLR4, and TLR6—has been demonstrated [43]. These different possible molecular associations appear to be the foundation of PAMP recognition and of their discrimination by macrophages. For example, the interaction of TLR2 with TLR1 is necessary for the recognition of the lipid configuration of the mycobacterial lipoprotein [44]. The combined expression of TLR2 and TLR4 allows the recognition of microbial components with different structures such as glycolipids or proteins [43]. In the case of *Pseudomonas aeruginosa* mannuronic acid polymers, TLR4 nonetheless had a predominant role, given that the alteration of TLR4 expression completely suppressed cytokine production, whereas release from TLR2 KO macrophages was only half of that seen with wild-type cells [45]. For yeast recognition, both TLR2 [25, 26] and TLR4 [26] have been shown to be involved. However, although TNF-α production could be inhibited by anti-TLR2 antibodies, no such alteration was observed in cells presenting defective expression of TLR4. Conversely, the chemokine response was shown to depend on TLR4 expression but did not involve TLR2 [26].

A differential cell response has already been shown for *C. albicans* but was more related to which *C. albicans*–derived molecules were involved. In particular, chemokine secretion is induced mainly by β-glucans, either β-1,6 [34] or β-1,3 [46] glucan. These components, which are present in the deepest layers of the *C. albicans* cell wall, are only accessible for interaction with macrophages when heat-killed yeasts are used, a procedure that removes surface components [47]. Glucans components have only limited activity on TNF-α production [46]. Zymosan, another yeast product that stimulates macrophages, is recognized by a TLR2-TLR6 heterodimer [48].

The complete involvement of TLR2 and the partial involvement of TLR4 and TLR6, with a balance between the respective importance of each receptor, was seen here for a single *C. albicans*–derived molecule. This molecule consists of PLM, which is a phylogenetically unique glycolipid composed of phytoceramide and long linear chains of mannose residues with unusual β-1,2 linkage types [16]. This composition confers to the molecule a highly specific structure [49]. β-1,2–oligomannosides present in PLM have been shown to interact with galectin-3 [37], whereas the ubiquitous α-mannosides expressed on other *C. albicans* molecules as well as by numerous microorganisms, among which nonpathogenic yeasts are prominent. Current innovative research concerns the pleiotropic activities of galectin-3 and its ability to modulate mammalian responses through its interaction with self-molecules. Considering the structure of PLM and the role of the lectin as a receptor for its glycan moiety, the hypothesis that galectin-3 could act as a coreceptor for TLR2 is currently being explored. The mecha-

nisms involved could be similar to those involved in LPS sensing and signaling, which require both the presence of a TLR (TLR4) and another secreted molecule, MD2 [50].

In conclusion, PLM, which is present at the cell-wall surface [12] and is shed from *C. albicans* in contact with host cells [15], directly initiates proinflammatory cytokine production through an interaction with TLR. The demonstration that TLR2 is the main receptor for *C. albicans* PLM may elucidate, at least in part, the triggering mechanism for this recently demonstrated receptor in *C. albicans*–induced proinflammatory cytokine production [26]. Together with its glycolipidic nature, these findings make it a new member of the PAMPs family. Because this molecule, which is specific to the most common commensal opportunistic yeast pathogen, has such a status, it is likely to have a key role in the early engagement of cells of innate immunity during the saprophytic-parasitic transition.

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