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Candida albicans Primes TLR Cytokine Responses through a Dectin-1/Raf-1–Mediated Pathway

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The immune system is essential to maintain homeostasis with resident microbial populations, ensuring that the symbiotic host–microbial relationship is maintained. In parallel, commensal microbes significantly shape mammalian immunity at the host mucosal surface, as well as systemically. *Candida albicans* is an opportunistic pathogen that lives as a commensal on skin and mucosa of healthy individuals. Little is known about its capacity to modulate responses toward other microorganisms, such as colonizing bacteria (e.g., intestinal microorganisms). The aim of this study was to assess the cytokine production of PBMCs induced by commensal bacteria when these cells were primed by *C. albicans*. We show that *C. albicans* and β-1,3-glucan induce priming of human primary mononuclear cells and this leads to enhanced cytokine production upon in vitro stimulation with TLR ligands and bacterial commensals. This priming requires the β-1,3-glucan receptor dectin-1 and the noncanonical Raf-1 pathway. In addition, although purified mannans cannot solely mediate the priming, the presence of mannosyl residues in the cell wall of *C. albicans* is nevertheless required. In conclusion, *C. albicans* is able to modify cytokine responses to TLR ligands and colonizing bacteria, which is likely to impact the inflammatory reaction during mucosal diseases. *The Journal of Immunology*, 2013, 190: 4129–4135.

Immediately after birth, the gastrointestinal tract is colonized by a diverse array of microbes, leading to a stable microbiota that is unique to each individual (1). The human body consists of trillions of somatic cells and harbors about 10 times more microorganisms in the intestines (2). The adult microbiota is composed of permanent members (up to 1000 species), as well as transient colonizers that are acquired from external sources. It consists of many prokaryotic and eukaryotic microbes, but bacterial species dominate. The most common anaerobic bacteria within the gastrointestinal tract are *Bacteroides*, *Bifidobacterium*, *Eubacterium*, *Fusobacterium*, *Clostridium*, and *Lactobacillus* species (3). These organisms are present in extremely high concentrations and have evolved to degrade a variety of dietary substances, simultaneously enhancing the host digestive efficiency, as well as their own nutrient supply (4).

In addition to their beneficial effects, gut microorganisms may be deleterious: when host defense is compromised, they may invade host tissues, which leads to mucosal and sometimes subsequent disseminated infection. A role for the gut flora in the pathogenesis of autoinflammatory diseases, such as Crohn's disease and ulcerative colitis, has also long been suggested (5). Hence, the immune system controls microbial composition; conversely, the gut microflora shapes immunity (6). Commensal microbes influence mucosal and systemic immunity, lymphoid structure development and epithelial function, T cell subsets, and innate lymph cells (6).

Candida albicans is primarily a human commensal of the skin and mucous membranes; however, under circumstances of disturbed host defense, it may become a pathogen (7, 8). The dual consequences of *C. albicans* colonization and/or infection suggest that this fungus might modulate the host immune response against other colonizing microorganisms. It was demonstrated that *C. albicans* triggers differential immune signaling upon interaction with either inflammatory or tolerogenic dendritic cells (9). The role of *C. albicans* in experimental colitis is controversial: on one hand it may activate dendritic cells in Peyer's patches (9), whereas on the other hand it may increase the severity of colitis (10, 11). Nevertheless, all of these studies were performed in mice, and it is not known whether *Candida* modulates inflammation in human primary cells.

In this study, using human primary cells, we investigated whether *C. albicans* is able to modulate cytokine responses to secondary stimuli, such as TLR ligands and colonizing microorganisms, by inducing either inflammation or tolerance. We established that *C. albicans* and, more specifically, β-1,3-glucan (β-glucan) can efficiently prime human immune cell responses to TLRs and bacteria, leading to enhanced cytokine production in vitro. By deciphering the receptor-signaling pathway involved (dectin-1/

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Abbreviations used in this article: β-glucan, β-1,3-glucan; RPMI, RPMI 1640 Dutch Modification.

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Raf-1), we also provide a possible novel therapeutic target in autoinflammatory disorders.

Materials and Methods

Blood samples

Blood was collected from healthy volunteers at Sanquin Bloodbank in Nijmegen, The Netherlands after obtaining informed consent. Human PBMCs were isolated from buffy coats.

Reagents

Ficoll-Paque (GE Healthcare) was used to isolate PBMCs by differential centrifugation. RPMI 1640 Dutch Modification (RPMI) (Sigma-Aldrich), supplemented with 1% gentamicin, 1% L-glutamine, and 1% pyruvate (Life Technologies, Nieuwekerk, The Netherlands) was used as culture medium. *Candida* β-glucan and mannan were isolated and purified as described previously (12, 13). Three sources of *C. albicans* mannan were tested: mannan serotype A, mannan isolated from *C. albicans* yeast grown in yeast extract/peptone/dextrose broth at 30˚C (pronase treated), and mannan isolated from *C. albicans* hyphae grown in serum at 37˚C (not treated with pronase). Pam_3Cys_4 (EMC microcollections) and LPS (*Escherichia coli* serotype 055:B5) were purchased from Sigma-Aldrich, with an additional purification step for LPS (14). Human dectin-1/CLEC7A mAb and isotype Ab were purchased from R&D Systems (Abingdon, U.K.).

Microorganisms

C. albicans ATCC MYA-3573 (UC 820), *och1*Δ, *och1*Δ/*och1*Δ/*OCH1*, *pmr*Δ, *pmr1*Δ/*pmr1*Δ/*PMR1*, *mnn4*Δ, *mnn4*Δ/*mnn4*Δ/*MNN4*, and the parental strain *C. albicans* NGY152 were grown overnight to yeast cells in Sabouraud broth at 37˚C. Cells were harvested by centrifugation, washed twice with PBS, and resuspended in culture medium (RPMI; ICN Biomedicals, Aurora, OH). *C. albicans* yeast was heat-killed for 30 min at 95˚C. To generate hyphae, yeast cells were grown at 37˚C in culture medium adjusted to pH 6.4 using hydrochloric acid. Hyphae were killed by exposure to 95˚C for 30 min and resuspended in culture medium to a hyphal inoculum size that originated from 1×10^6/ml. The *OCH1* gene was disrupted using the ura-blaster method (15), and the reintegrant strain was constructed as previously described (16). Creation of the *mnn4* null mutant and of its reintegrant *mnn4*Δ/*mnn4*Δ/*MNN4* control strain was performed as described (17). The *PMR1* gene was disrupted and reintroduced in *pmr1*Δ/*pmr1*Δ/*PMR1* as described (18). Three common colonizing bacteria of the intestines or the skin were chosen: *E. coli* ATCC 35218 was grown overnight in culture medium, washed three times with PBS, and heat-killed for 60 min at 80˚C; *Staphylococcus aureus* strain Seattle 1945 (ATCC 25923) was grown overnight in culture medium, washed twice with cold PBS, and heat-killed for 30 min at 100˚C; and *Bacteroides fragilis* ATCC 25285 was grown as before but was heat-killed for 30 min at 95˚C.

In vitro cytokine production

The PBMC fraction was obtained by density centrifugation of diluted blood (1 part blood:1 part pyrogen-free saline) over Ficoll-Paque (GE Healthcare). PBMCs were washed twice in saline and resuspended in culture medium. Cells were counted with a Beckman Coulter Z1 Particle Counter and adjusted to 5×10^6 cells/ml. PBMCs (5×10^6) were added at a volume of 100 μl/well in round-bottom 96-well plates (Greiner, Nürnberg, Germany). Cells were incubated with one of the first stimuli for 24 h and reincubated for another 24 or 48 h with one of the second stimuli. As a priming stimulus, we used 50 μl either heat-killed *C. albicans* yeast or hyphae at two concentrations (1×10^3/ml or 1×10^4/ml), β-glucan (100 μg/ml), mannan (100 μg/ml), three *C. albicans* mutants and their corresponding reintegrants: heat-killed *och1*Δ null mutant, *pmr1*Δ null mutant, *mnn4*Δ null mutant, *och1*Δ/*och1*Δ/*OCH1*, *pmr1*Δ/*pmr1*Δ/*PMR1* and *mnn4*Δ/*mnn4*Δ/*MNN4* revertant strains (1×10^4/ml), as well as their parental strain NGY152 (1×10^4/ml). After 24 h, cells were subjected to a second stimulation with various stimuli, in a volume of 50 μl. The second stimulus was one of the following: LPS (10 ng/ml), Pam_3Cys_4 (10 μg/ml), heat-killed *E. coli* (1×10^8/ml), heat-killed *S. aureus* (1×10^7/ml), or heat-killed *B. fragilis* (1×10^7/ml) in a final volume of 200 μl. After 24 or 48 h, supernatants were collected and stored at −20˚C until assayed. To determine whether our priming reagents were endotoxin free, we performed a series of experiments in the presence of polymyxin B. Prior to priming, the reagents were incubated for 3 h with polymyxin B (2 μg/ml).

To investigate whether priming could be blocked, we preincubated PBMCs for 1 h with dectin-1/CLEC7A Ab (dectin-1 antagonist; 10 μg/ml) or with the isotype control (10 μg/ml), Syk inhibitor (50 nM) or Raf-1 inhibitor (1 μM), before priming with β-glucan. After this, cells were treated with either RPMI or β-glucan (100 μg/ml) and incubated for 24 h, followed by a second stimulation with LPS (10 ng/ml) for an additional 24 h. Cytokines were measured after the second stimulation.

Cytokine assay

The concentrations of TNF-α and IL-1β (R&D Systems) and IL-6 and IL-10 (Sanquin, Amsterdam, The Netherlands) were measured in cell culture supernatants using ELISA, according to the manufacturer's instructions. Proinflammatory cytokines TNF-α, IL-1β, and IL-6 were measured at 24 h after the second stimulation, whereas the anti-inflammatory cytokine IL-10 was measured at 48 h after the second stimulation.

Statistical analysis

Results from at least two sets of experiments with a minimum four volunteers were pooled and analyzed with SPSS. Data are given as mean ± SEM. The paired Wilcoxon test was used to compare differences between the effect of a particular stimulus and the RPMI medium control. The level of significance was set at $p < 0.05$.

Results

C. albicans *primes the cytokine responses of cells restimulated with TLR ligands and colonizing bacteria*

To investigate the effect of *C. albicans* on the cytokine responses to colonizing bacteria, PBMCs from healthy donors were first exposed to low doses of *C. albicans.* After 24 h, cells were restimulated with common colonizing bacteria of the skin or gut or with pure components of the bacterial or fungal cell wall (Fig. 1A). Exposure to either *C. albicans* yeast or hyphae enhanced the production of TNF-α, IL-6, and IL-1β (Fig. 1B, 1C) of PBMCs incubated with a second bacterial stimulus, such as *E. coli*, *S. aureus*, or *B. fragilis*. This priming effect was also observed when bacterial cell wall components, such as LPS, found in the outer membrane of Gram-negative bacteria, and the lipopeptide analogs of the N terminus of bacterial lipoprotein, Pam_3Cys_4, were used as a second stimulus. Notably, the increased production of proinflammatory cytokines did not correlate with a decrease in the anti-inflammatory cytokine IL-10 (Fig. 1B, 1C). A 10-fold lower concentration of *C. albicans* yeast or hyphae was also able to prime but to a lesser extent (~2-fold less).

Mannans do not prime cytokine production by themselves but are essential for potentiation by C. albicans

The outer layer of the cell wall of *C. albicans* is enriched with mannoproteins, whereas the inner layer is composed of chitin and β-glucan and β-1,6-glucan (19). Mannosylated proteins and phospholipomannan of *C. albicans* are known to induce proinflammatory cytokines (20, 21). Therefore, we assessed the priming effect of pure mannans using the in vitro experimental setting depicted in Fig. 1A. We tested three purified preparations of *C. albicans* mannan as priming stimuli; none produced a priming effect compared with the nonprimed control (Fig. 2).

Because mannans may potentiate priming by other cell wall components, we used a genetic complementary approach to investigate the role of specific mannosyl residues. The *C. albicans och1* null mutant has a defect in *N*-linked mannosylation (Fig. 3A), resulting in a 77% reduction of cytokine production in direct stimulations (19). In contrast, the *C. albicans mnn4* mutant, defective in tertiary branch mannosylphosphorylation (Fig. 3A) (17, 22), has no effect on the direct induction of cytokine production. *C. albicans pmr1* null mutant has a defect in phosphomannosylation, as well as in *O*- and *N*-linked glycosylation (18) (Fig. 3A). In our preincubation model, both *C. albicans och1* and *pmr1* null mutants had a partial defect in priming properties and could not potentiate the production of proinflammatory cytokines to the same extent as the parental strain (Fig. 3B, 3C). Of note, the priming effect was restored when cells were exposed to the

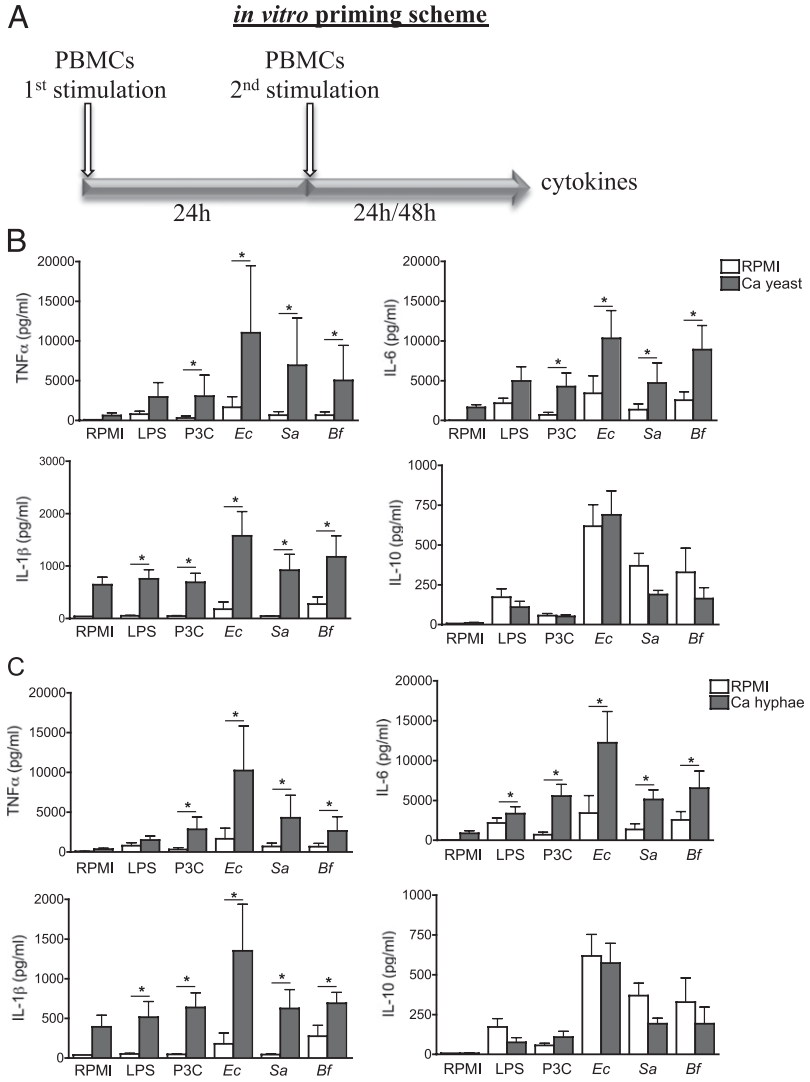

FIGURE 1. *C. albicans* primes the cytokine responses of cells restimulated with TLR ligands and colonizing bacteria. (**A**) In vitro priming scheme. PBMCs were incubated with *C. albicans* (priming stimulus) for 24 h at 37˚C. Thereafter, bacteria found in the human microbiota, as well as their cell wall pure components, were added (second stimuli) for an additional 24 or 48 h. PBMCs were exposed to RPMI or heat-killed *C. albicans* yeast (**B**) or hyphae (**C**) (1×10^4 cells/ml). After 24 h, PBMCs were exposed to several pure ligands or heat-killed bacteria. Cytokines were measured in supernatants 24 h (TNF-α, IL-1β, and IL-6) and 48 h (IL-10) after the second stimulation ($n = 6$; three independent experiments). Bars indicate mean + SEM. $*p < 0.05$ versus RPMI–primed cells, Wilcoxon nonparametric test for two related samples. *Bf*, *B. fragilis*; *Ec*, *E. coli*; P3C, Pam_3Cys_4; *Sa*, *S. aureus*.

reconstituted strains, *och1*Δ/*och1*Δ/*OCH1* and *pmr1*Δ/*pmr1*Δ/*PMR1* (Fig. 3B, 3C). The *mnn4* null mutant was still able to prime cytokine production to the same extent as the parental strain *C. albicans* NGY152, and the same was true for the reconstituted strain *mnn4*Δ/*mnn4*Δ/*MNN4* (Fig. 3D). Altogether, these results show that, although pure mannans do not prime for the production of proinflammatory cytokines, *O*- and, especially, *N*-linked mannosyl proteins at the surface of *C. albicans* are nevertheless required for the proper priming.

β-glucan primes the response to components of human microbiota through dectin-1 and Raf-1

Although β-glucan is a component of the inner layer of the *Candida* cell wall, it is a potent inducer of cytokines through its interaction with dectin-1 (23). Therefore, we assessed the effect of β-glucan in the priming model. Incubation of PBMCs with purified β-glucan from *C. albicans* was able to exert a priming effect similar to that obtained with *C. albicans* whole organism (Fig. 4A). The effect was most prominent for TNF-α and IL-1β and less so for IL-6. The β-glucan priming led to a decreased amount of the anti-inflammatory cytokine IL-10 induced by the secondary stimuli (Fig. 4A).

When we blocked the dectin-1 receptor with an anti–dectin-1 Ab before priming with β-glucan, the priming effect was reduced considerably, whereas the isotype control had no effect (Fig. 4B). We completed the analysis using PBMCs from two sisters with congenital dectin-1 deficiency (24). In these experiments β-glucan priming was completely abolished, highlighting the importance of the dectin-1 receptor in mediating the β-glucan–dependent priming of cytokine production upon exposure to TLR ligands and colonizing bacteria (Fig. 4C). Dectin-1 signaling is primarily mediated by Syk/CARD9 (25). The inhibition of Syk kinase before β-glucan stimulation did not affect the priming and subsequent enhancement of TNF-α release upon LPS restimulation (Fig. 4D). However, inhibiting the noncanonical serine-threonine Raf-1 kinase (26) significantly decreased the priming effect (Fig. 4E). Altogether, these results show that β-glucan, a major component of the cell wall of *C. albicans*, exerts a strong priming effect toward restimulation with TLR ligands and colonizing bacteria and that this effect is mediated through the β-glucan receptor dectin-1 and the noncanonical Raf-1 kinase pathway.

Because live *C. albicans* cell walls consist of β-glucan masked by a layer of mannosylated proteins, it is possible that the simultaneous priming of PBMCs with β-glucan and mannan induces the production of proinflammatory cytokines to the same extent as does whole *Candida*. However, the priming effect of the two major *C. albicans* cell wall components, β-glucan and mannan, was not different from that of β-glucan alone (Fig. 5). These results underscore our observation that the priming of *C. albicans* is due to β-glucan and not to mannan.

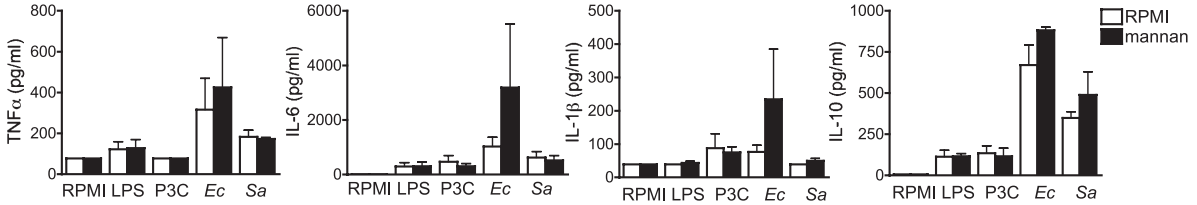

FIGURE 2. By themselves, mannans do not prime cytokine production, but they are essential for potentiation by *C. albicans*. PBMCs were exposed to either RPMI or *C. albicans* mannan isolated from *C albicans* hyphae grown in serum at 37˚C at a concentration of 100 μg/ml. After 24 h, PBMCs were restimulated with several pure ligands or heat-killed bacteria. Cytokines were measured in supernatants 24 h (TNF-α, IL-1β, and IL-6) and 48 h (IL-10) after the second incubation. Three types of *C. albicans* mannan were tested, but none of them induced priming compared with the nonprimed control ($n \geq 5$; two independent experiments). No statistical differences were detected using the Wilcoxon nonparametric test for two related samples. *Ec*, *E. coli*; P3C, Pam_3Cys_4; *Sa*, *S. aureus*.

Priming is not due to endotoxin contamination

Prior to priming, all stimuli (RPMI, β-glucan, and mannan) were incubated with cell culture medium (control) or with polymyxin B for 3 h at 37˚C. Thereafter, PBMCs were primed for 24 h and then restimulated with LPS, Pam_3Cys_4 or cell culture medium only (RPMI) for an additional 24 h (Supplemental Fig. 1). Polymyxin B treatment of β-glucan did not affect its ability to prime PBMCs, excluding the effect of endotoxin contamination in the described effect. Mannan was still not able to prime, either with or without polymyxin B. In addition, β-glucan and polymyxin B priming was

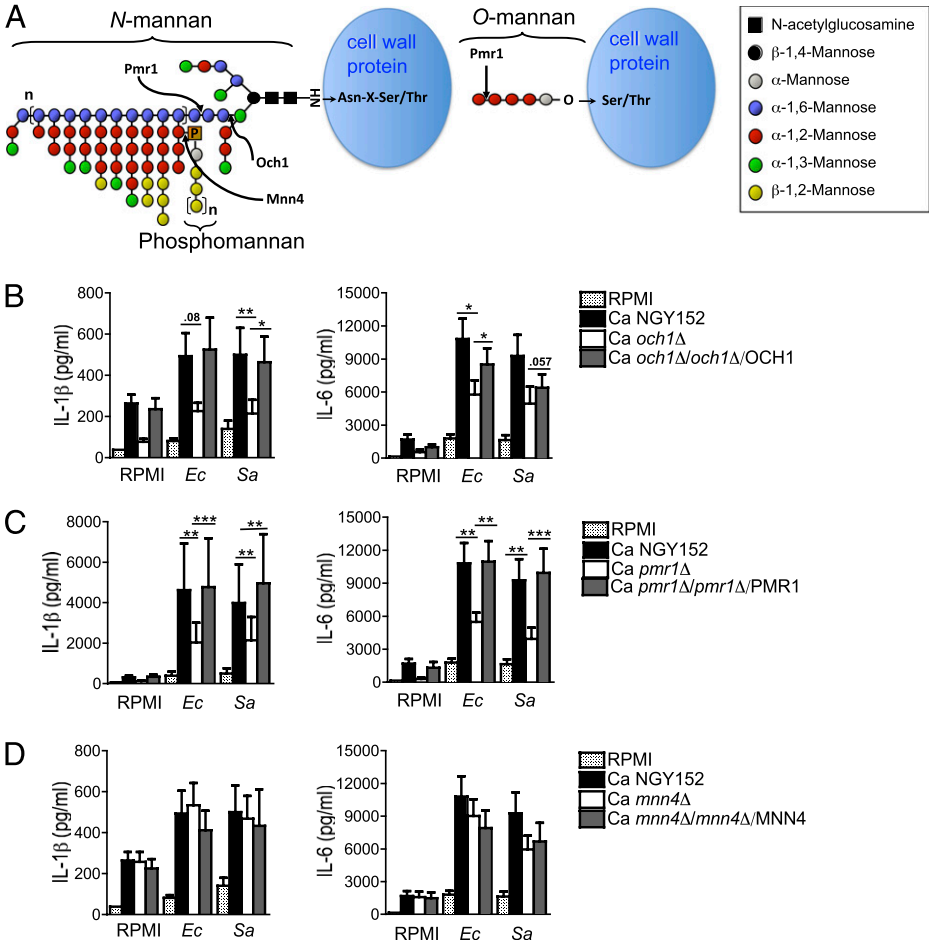

FIGURE 3. Mannosylation of fungal cell wall proteins is involved in the potentiation of cytokine production. (**A**) Schematic diagram representing the activity of Och1, Pmr1, and Mnn4 enzymes on *C. albicans* cell wall glycosylation. For *N*-mannosylation, the Och1 mannosyltransferase mediates the addition of the first α-1,6-mannose to the core *N*-mannan structure. Two α-1,6-mannose residues form the backbone onto which α-1,2-mannose– and α-1,3-mannose–linked mannose residues are subsequently attached by different enzymes to form the *N*-linked outer chain. Phosphomannan is attached to this outer chain via a phosphodiester bond, which requires Mnn4. Pmr1 is not a glycosyltransferase and does not add any mannans directly but exerts its effects on both *N*-mannosylation and *O*-mannosylation. The arrows represent where truncations in *N*-mannosylation and *O*-mannosylation occur. (**B**–**D**) PBMCs were exposed to NGY152, *och1*Δ, *pmr1*Δ, *mnn4*Δ, *och1*Δ/*och1*Δ/*OCH1*, *pmr1*Δ/*pmr1*Δ/*PMR1*, or *mnn4*Δ/*mnn4*Δ/*MNN4* (10^4 cells/ml). After 24 h, PBMCs were restimulated with heat-killed bacteria. Cytokines (IL-1β and IL-6) were measured in supernatants 24 h after the second incubation ($n = 10$; four independent experiments). Bars indicate mean ± SEM. All *C. albicans* strains induced a significant increase in proinflammatory cytokine production compared with nonprimed (RPMI) cells, whereas *och1*Δ and *pmr1*Δ null mutants resulted in significantly less priming of cytokine production compared with the parental strain or their corresponding reconstituted strains. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, Wilcoxon nonparametric test for two related samples. *Ec*, *Escherichia coli*; *Sa*, *S. aureus*.

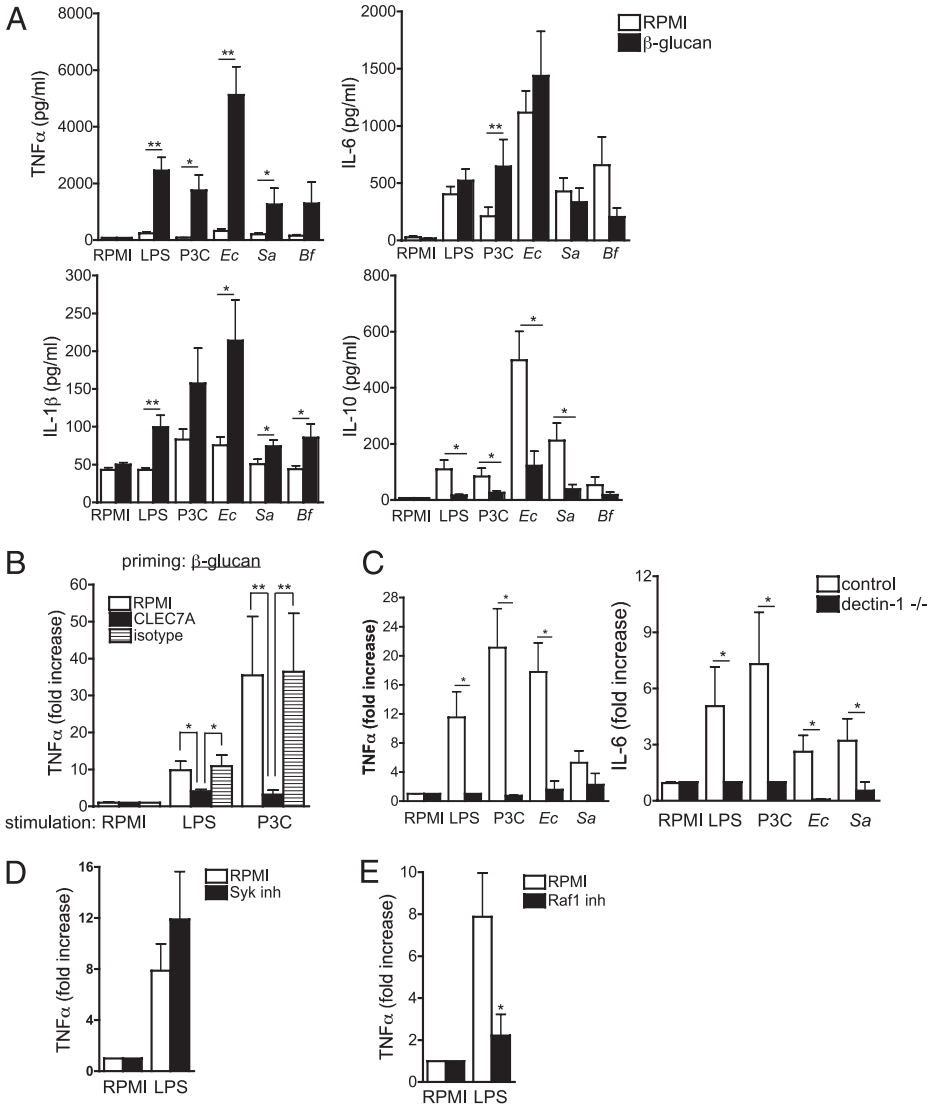

FIGURE 4. β-glucan primes the response to components of human microbiota. (**A**) PBMCs were incubated with RPMI or β-glucan (100 μg/ml). After 24 h, PBMCs were reincubated with pure ligands or heat-killed bacteria. Cytokines were measured in supernatants 24 h (TNF-α, IL-1β, and IL-6) and 48 h (IL-10) after the second stimulation ($n = 11$; five independent experiments). (**C**) PBMCs from 16 healthy volunteers and 2 dectin-1–deficient sisters (24) were incubated with RPMI and β-glucan (100 μg/ml) for 24 h, followed by a second stimulation with either TLR ligands or whole bacteria for an additional 24 h. PBMCs were incubated with RPMI, CLEC7A Ab, and the isotype control (10 μg/ml) (**B**), Syk inhibitor (1 μM) (**D**), or Raf-1 inhibitor (50 nM) (**E**). After 1 h of incubation, cells were treated either with RPMI or β-glucan (100 μg/ml) and, after an additional 24 h, restimulated with LPS. For (B), $n = 7$; three independent experiments; for (D) and (E), $n = 10$; five independent experiments. Bars indicate mean ± SEM. $*p < 0.05$, $**p < 0.01$ versus RPMI–stimulated cells, Wilcoxon nonparametric test for two related samples. *Bf*, *B. fragilis*; *Ec*, *E. coli*; P3C, Pam_3Cys_4; *Sa*, *S. aureus*.

completely abrogated when PBMCs were restimulated with LPS; this was not the case for the same mix and restimulation with Pam_3Cys_4.

Discussion

In the current study, we analyzed the capacity of the human commensal yeast *C. albicans* to modulate the cytokine response of human primary cells to bacteria that usually colonize the skin and mucosal surfaces. *C. albicans*, either yeast or hyphae, primes the cells for enhanced proinflammatory cytokine production when they are exposed to a secondary bacterial stimulus. This priming activity is dependent on carbohydrate components of the cell wall of *Candida*, with an important role for the recognition of β-glucan by the dectin-1/Raf-1 pathway.

An important aspect is which molecular pattern of *C. albicans* is responsible for this increased immune activity. Combining pu-

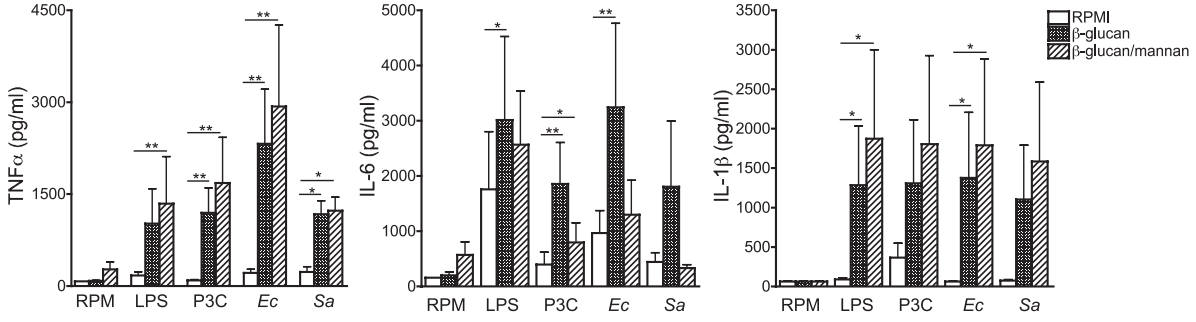

FIGURE 5. Priming with a combination of β-glucans and mannans. PBMCs were incubated simultaneously with β-glucan and mannan (100 μg/ml); after 24 h, cells were stimulated either with TLR ligands or with whole bacteria for an additional 24 h ($n = 7$; three independent experiments). Bars indicate mean ± SEM. $*p < 0.05$, $**p < 0.01$ versus RPMI–stimulated cells, Wilcoxon nonparametric test for two related samples. *Ec*, *E. coli*; P3C, Pam_3Cys_4; *Sa*, *S. aureus*.

rified cell wall components and genetic approaches, we established that fungal mannan itself does not prime PBMCs for the production of cytokines. Surprisingly, however, neither the *och1* nor the *pmr1* null *C. albicans* mutant was able to prime the production of proinflammatory cytokines at the same extent as was wild-type *C. albicans* or parental strain NGY152, whereas the use of a reconstituted strain entirely restored the priming effect. Thus, the presence of the *O*- and *N*-linked mannosyl proteins at the *Candida* cell surface is required for proper priming, supposedly by aiding cell priming by an additional cell wall component. Of note, although, under aqueous conditions, long chains of purified β-glucan form a right-handed triple helical complex that is required for the proper binding to pattern recognition receptors (27–29), purified mannan might not have the required structure to be recognized by its receptor, offering an alternative explanation for the lack of priming induced by pure mannans. Different biological effects of mannans were reported, including induction of proinflammatory cytokines (19, 30) and of IL-10 (31). It was shown that when *C. albicans* mannoprotein was administered to mice before or during immunization with viable *C. albicans*, it downregulated mannan-specific delayed hypersensitivity (32), whereas mannoproteins confer protection to candidiasis and might contain epitopes involved in this protective response (32). In this study, we show that mannans incorporated in *Candida* cell wall have adjuvant activity for the priming effect exerted by this yeast.

In search of the main component of the *Candida* cell wall that induces priming, we demonstrate that β-glucan can mimic the priming effect obtained upon *C. albicans* preincubation. This effect of β-glucan is in line with previous studies showing that β-glucan is an immunostimulant and, moreover, displays antitumor activity (33, 34). The receptor mediating the priming effects of β-glucans is dectin-1, as shown by experiments with cells genetically deficient for this receptor. Downstream signaling occurs via two pathways: the canonical Syk/CARD9 and the noncanonical serine-threonine kinase Raf-1 (25, 26, 35). We found that inhibition of Syk kinase, prior to exposure to β-glucan, did not influence the priming activity of β-glucan. In contrast, the incubation of PBMCs with a Raf-1 inhibitor prior to first stimulation significantly reduced the effect. This observation distinguishes the direct effect of dectin-1 stimulation (Syk dependent) from the priming effect mediated by dectin-1 (Raf-1 dependent). Indeed, a previous study showed a dual intracellular signaling upon dectin-1 activation, in which the Raf-1 pathway integrates with the Syk pathway at the level of NF-κB (35). Because priming is induced by purified TLR ligands, which are important components of bacteria, we tend to conclude that the priming induced by *Candida* and β-glucans acts on TLR-dependent–signaling pathways.

C. albicans is a common commensal of the intestinal tract, and it was reported to increase the severity of intestinal inflammation in murine models of colitis (10, 11). The β-glucan receptor dectin-1 is involved in this effect, and polymorphisms in this receptor have been associated with the severity of ulcerative colitis (11) but not with the susceptibility to ulcerative colitis or Crohn's disease (36). Little is known about the mechanisms through which *C. albicans* may influence autoinflammatory diseases, such as Crohn's disease. Although simultaneous stimulation of cells with *Candida*-derived polysaccharides and TLR ligands is known to be synergistic for cytokine induction, it is not known whether prestimulation of immune cells with *Candida* can prime or tolerize (as in the case of TLR stimulation with LPS or Pam_3Cys_4) the cells for subsequent stimulation with colonizing bacteria. In the current study, we demonstrate that preincubation of human primary cells with *C. albicans* primes them for the production of cytokines, and this amplification of inflammation may represent one mechanism for the increased severity of colitis induced by *Candida*. The priming effect found in this study may be relevant for these observations. Whether this effect of *Candida* is also relevant for inflammatory bowel disease in humans is not clear. However, because Abs toward *C. albicans* were shown to be associated specifically with Crohn's disease, one may speculate that *Candida* plays a role in this disease (37). It is attractive to hypothesize that this has to do with the priming effect described in this article.

Chronic stimulation of host immune cells by microorganisms may result in an enhanced functional state and, hence, increased resistance to infection or in immunosuppression (or tolerance). The latter may imply protection against tissue damage. Understanding the interplay between these two strategies may allow us to define how fungi adapt to the human immune system. It was proposed that *C. albicans* may either promote tolerance (9) or amplify inflammation and contribute to disease activity in Crohn's disease (11). In the current study, *C. albicans* did not induce tolerance, but it promoted the proinflammatory response of human primary cells to colonizing bacteria. Such priming may be related to the epigenetic reprogramming of *C. albicans* described in monocytes (38). Thus, the fungal microbiota may actively contribute to the state of host defense at mucosal sites. When leukocytes at the mucosal sites previously primed by *C. albicans* encounter potentially pathogenic colonizing bacteria, the enhanced state of the host response may potentiate the defense (e.g., by recruitment and activation of effective immune cells and by induction of diffusion in mucosal sites).

In conclusion, we demonstrate that chronic exposure of primary human immune cells to *C. albicans* primes them for subsequent stimulation with TLR ligands and colonizing microorganisms, a mechanism that could explain the effects of this fungus on inflammatory bowel diseases. By identifying dectin-1/Raf-1 as one of the pathways responsible for these effects, we provide a potential therapeutic target in autoinflammatory diseases, such as Crohn's disease. Further studies are warranted to assess this hypothesis.

Disclosures

The authors have no financial conflicts of interest.

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