MAJOR ARTICLE

Colonization of Mice by *Candida albicans* Is Promoted by Chemically Induced Colitis and Augments Inflammatory Responses through Galectin-3

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Background. Little is known about the relationship between colonic inflammation and *Candida albicans* colonization. Galectin-3 (Gal-3) is an intestinal lectin that binds to specific *C. albicans* glycans and is involved in inflammation.

Methods. Colitis was experimentally induced in wild-type and *Gal3*$^{-/-}$ mice using dextran sulfate sodium (DSS) before oral administration of *C. albicans.* Yeast recovered from stools was quantified. The presence of yeast and inflammation were evaluated in sections of colon by histologic examination, quantification of myeloperoxidase (MPO) activity, and by gene expression for cytokines and innate immune receptors. Serum from mice was collected for determination of anti-yeast mannan antibodies, including anti–*Saccharomyces cerevisiae* antibodies (ASCA), which are biomarkers of an inflammatory bowel disease.

Results. Inflammation strongly promoted *C. albicans* colonization. Conversely, *C. albicans* augmented inflammation induced by DSS, as assessed by histologic scores, MPO activity, and tumor necrosis factor (TNF)-α and Toll-like receptor (TLR)-2 expression. *C. albicans* colonization generated ASCA. The absence of Gal-3 reduced DSS inflammation and abolished the response of TLR-2 and TNF-α to *C. albicans* colonization.

Conclusions. DSS-induced colitis provides a model for establishing *C. albicans* colonization in mice. This model reveals that *C. albicans* augments inflammation and confirms the role of Gal-3 in both inflammation and the control of host responses to *C. albicans.*

Candida albicans colonizes the human gut, which is the primary source of yeast for invasive infections in hospitalized, immunocompromised patients [1, 2]. Recently, a link was established between *C. albicans* and inflammatory bowel diseases through demonstration that this endogenous yeast could induce anti-oligomannose antibodies (anti–*Saccharomyces cerevisiae* antibodies, or ASCA), which are markers of Crohn disease [3].

However, little is known about the molecular relationships between *C. albicans* and the host receptors that govern colonization, tolerance, inflammation, and invasion at the gut level [4]. Experimental studies in mice are limited by the fact that *C. albicans* is not a natural part of the murine digestive flora [5, 6]. Establishment of colonization therefore requires the use of either infant [5, 7] or antibiotic-treated adult mice [8–10]. In this study, we used dextran sulfate sodium (DSS)-induced colitis in mice, a model that is widely used to study the relationship between inflammation and the endoluminal microbiota [11], but which had never been adapted to *C. albicans.*

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With this model, we explored the effect of *C. albicans* colonization on inflammation, at both the macroscopic and molecular levels. We examined the involvement of innate immunity mediators and receptors, including galectin-3 (Gal-3), which is a pleiotropic lectin that participates in inflammation but has also been described as a specific receptor for *C. albicans* [12]. A recent study has confirmed that Gal-3 binds to *C. albicans* and that high levels of Gal-3 can be detected in human tissues infected by *C. albicans* [13]. Simultaneously, it was demonstrated that *C. albicans* promoted the association of Gal-3 with the innate receptor Toll-like receptor (TLR)-2 to induce a macrophage response, which included secretion of the proinflammatory cytokine tumor necrosis factor (TNF)-α [14].

Using wild-type (WT) and *Gal3*–deficient mice (hereafter, *Gal3*$^{-/-}$ mice), the objectives of this study were to investigate the following: (1) the effect of DSS-induced inflammation on *C. albicans* colonization; (2) the effect of *C. albicans* colonization on inflammation, as measured by histologic scores, neutrophil infiltration, gene expression of pathogen recognition receptors, and cytokines; and (3) the role of Gal-3 in the regulation of inflammation induced by *C. albicans,* specifically in relation to TLR-2 and TNF-α secretion and ASCA generation.

MATERIALS AND METHODS

Animals. All animal experiments conformed to the Ministère de l'Agriculture et de la Forêt Resolution on the use of animals in research and were approved by the Subcommittee on Research Animal Care of the Regional Hospital Center of Lille (protocol 2003–35). The production of *Gal3*$^{-/-}$ mice by use of gene-targeting technology has been described elsewhere [15]. As controls, age- and sex-matched WT (C57BL/6) littermates were used. Mice were maintained by Charles River Laboratories (France). Six- to 8-week-old female mice were used in this study. Animals were housed in groups and had free access to regular rodent chow and tap water.

Induction of colitis,* C. albicans *administration, and experimental design. Colitis was experimentally induced in mice by administration of 5% DSS (molecular weight, 36–50 kDa; MP Biomedicals) in drinking water from day 1 to day 7. Mice were inoculated on day 3 by single gavage with 200 μL of PBS containing 10^7 live cells of *C. albicans* SC5314 reference strain [16]. No mortality was observed during the 7 days that DSS was administered.

WT and *Gal3*$^{-/-}$ mice were each distributed into 1 control group and 3 experimental groups. A group of healthy mice was used as controls (CTL) (5 WT and 6 *Gal3*$^{-/-}$ mice). A second group of mice was gavaged orally with *C. albicans* without any other treatment (CaCTL) (5 WT and 8 *Gal3*$^{-/-}$ mice). A third group was treated with DSS (DSS) (5 WT and 7 *Gal3*$^{-/-}$ mice). A fourth group was treated with DSS and gavaged orally with *C. albicans* (CaDSS) (6 WT and 7 *Gal3*$^{-/-}$ mice).

At day 14, the animals were sacrificed by cervical dislocation. Blood was collected by cardiac puncture and serum samples were stored at −20°C until use. The entire colon from the cecum to the anus was removed, and different anatomic sections were stored at −80°C until use.

Evaluation of* C. albicans *colonization. The presence of yeast in the intestinal tract was evaluated by performing plate counts for cultures of feces collected from each animal on day 14. The fecal samples were suspended in 1 mL of PBS, ground in a glass tissue homogenizer and plated onto Sabouraud dextrose agar containing 500 mg/L amikacin sulfate. Colonies of *C. albicans* were counted after 48 h incubation at 37°C. The results were noted as cfu/10 μL, which corresponded to cfu/10 μg of feces.

Detection of ASCA. Serum antibodies against *C. albicans* and *S. cerevisiae* mannan were detected using ELISA tests [17, 18], which were initially designed to detect human antibodies. Antigens consisted of mannan extract from *C. albicans* VW32 yeasts for detection of anti–*C. albicans* mannan antibodies or *S. cerevisiae* SU1 for detection of ASCA. In brief, plates were coated with 100 μL of mannan at a concentration of 1 μg/mL of sugars in sodium carbonate buffer (60 mmol/L; pH 9.6). After incubation and washing in Tris-NaCl-Tween (TNT) (Tris-HCl, 50 mmol/L; NaCl, 150 mmol/L; Tween, 0.05%; pH 7.5), 100 μL of 1:100 diluted serum was added to the coated wells. Peroxidase-labeled anti-mouse immunoglobulins (G, A, M) (Zymed Laboratories) were diluted 1:1000 in TNT. Absorbance was read at 450 nm (reference filter, 620 nm) in a microplate reader (Bio-Rad Laboratories) after addition of tetramethylbenzydine. Results were expressed as optical density (OD).

Microscopy. Rings of the transverse part of the colon were fixed overnight in 4% paraformaldehyde-acid and embedded in paraffin for histologic analysis. Cross-sections (4 μm thick) were stained with May-Grünwald-Giemsa stain (Merck). Histologic scores were evaluated by 2 independent, blinded investigators who observed 2 sections per mouse at magnifications of $\times$10 and $\times$100. The scores were determined in accordance with Siegmund et al. [19], and the sections were evaluated for the following 2 subscores: (1) a score for the presence and confluence of inflammatory cells, including neutrophils, in the lamina propria, and submucosal or transmural extension; and (2) a score for epithelial damage, focal lymphoepithelial lesions, mucosal erosion and/or ulceration, and extension to the bowel wall. The 2 subscores were added together, and the combined histologic score ranged from 0 (no changes) to 6 (extensive cell infiltration and tissue damage).

Fluorescence staining of* C. albicans *in situ. Paraffin-embedded sections were dewaxed and rehydrated with PBS. The tissue sections were then blocked in 1% bovine serum albumin (BSA) diluted in PBS for 30 min at room temperature. Slides were washed in PBS and sections were incubated for 1 h at room temperature with monoclonal antibody 5B2, which reacts with β-linked oligomannose epitopes of the *C. albicans* cell wall, di-

Table 1. Mouse oligonucleotide sequences.

Primer	Sequence (5'→3')
β-actin S	TCA CCC A CA CTG TGC CCA TCT ACG A
β-actin AS	CAG GGG AAC CGC TCA TTG CCA ATG
TNF-α S	TGG GAG TAG ACA AGG TAC AAC CC
TNF-α AS	CAT CTT CTC AAA ATT CGA GTG ACA A
IL-1β S	CAA CCA ACA AGT GAT ATT CTC CAT
IL-1β AS	GAT CCA CAC TCT CCA GCT GCA
TLR-2 S	ACG GGG CCA TCC TTG TT
TLR-2 AS	TTA TCT TGC GCA GTT TGC AGA A
TLR-4 S	GAC CAA GCC TTT CAG GGA ATT
TLR-4 AS	GGA CGT GTA AAC CAG CCA GGT
NOD-2 S	GGA AAC AAC ATT GGC AGC AT
NOD-2 AS	TCT TGA GTC CTT CTG CGA GA

NOTE. S, sense; AS, antisense.

luted 1:100 in PBS [20]. After 3 washes with PBS, sections were incubated with fluorescein isothiocyanate–conjugated goat anti-mouse IgM (Zymed Laboratories) for 60 min at 37°C. The sections were then washed with PBS and counterstained with PBS containing 0.02% Evans blue. The sections were examined by immunofluorescence microscopy (Leica Microsystems AG).

Determination of tissue myeloperoxidase (MPO) activity. Colon tissue samples included tissue from the mid- to distal colon (adjacent to the tissue used for histology). Samples were rinsed with cold PBS, blotted dry, and immediately frozen in liquid nitrogen. The samples were stored at −80°C until MPO activity was estimated by the O-dianisidine method [21, 22]. In brief, tissue samples were weighed, suspended (10%, wt/vol) in 50 mmol/L potassium phosphate buffer (pH, 6.0) containing 0.5% hexadecyltrimethylammonium bromide (0.1 g/20 mL potassium phosphate), and homogenized. A 1-mL sample of the homogenate was sonicated for 30 s. The sample was then centrifuged at 14,000 g for 15 min at 4°C. The MPO level in the supernatant was determined by adding O-dianisidine dihydrochloride and H_2O_2 solution (O-dianisidine, 0.167 mg/mL; potassium phosphate buffer, 50 mmol/L, $H_2O_2$0.0005%). The absorbance was read at 450 nm using a 96-well microplate reader. One unit of MPO activity was defined as the quantity able to convert 1 μmol of H_2O_2 to water in 1 minute at 20°C and was expressed as units/mg protein. Total protein concentrations were quantified using a Bio-Rad DC Protein assay kit.

Real-time mRNA quantification. Total RNA was isolated from colon samples using a NucleoSpin RNA II kit (Macherey-Nagel) following the manufacturer's instructions, with 20–50 units of DNase I (RNase-free) at 37°C for 30 min to avoid contamination with genomic DNA. RNA quantification was performed by spectrophotometry (Nanodrop; Nyxor Biotech). Reverse transcription of mRNA was carried out in a final volume of 26 μL from 1 μg total RNA using 300 U Moloney murine leukemia virus reverse transcriptase (Invitrogen) according to the manufacturer's instructions with 500 ng oligo (dT) 12–18 and 50 U ribonuclease inhibitor (RNase-Out; Promega). PCR was performed using an ABI 7000 prism sequence detection system (Applied Biosystems) with SYBR green (Applied Biosystems). Amplification was carried out in a total volume of 25 μL containing 0.5 μL of each primer (table 1) and 1 μL of cDNA prepared as described above. SYBR green dye intensity was analyzed using Abiprism 7000 SDS software. All results were normalized to the housekeeping gene β-actin.

Statistics. Data were expressed as the mean ± SE for each group. All comparisons were analyzed by the Mann-Whitney *U* test. Statistical analyses were performed using StatView statistical software (version 4.5; SAS Institute). Differences were considered significant when $P < .05$.

RESULTS

DSS administration and* C. albicans *colonization in WT and* Gal3$^{-/-}$ *mice. The persistence of *C. albicans* in the intestinal tract of mice with or without DSS administration was evaluated on day 14 by counting colony-forming units of yeast in feces. In WT and *Gal3*$^{-/-}$ mice inoculated with *C. albicans,* very few colony-forming units were recovered from stools in the absence of DSS treatment. In contrast, in DSS-treated mice, significantly higher numbers of colony-forming units were recovered from stool samples ($P < .05$) (figure 1). No significant difference was observed between WT and *Gal3*$^{-/-}$ mice.

The presence of yeast in the gut was examined by immunofluorescence staining of yeast with an anti–*C. albicans* β-mannose monoclonal antibody in colon sections from sacrificed mice. The results obtained showed that the presence of yeast in the stools was indeed associated with the presence of large quantities of *C. albicans* blastoconidia, either in the lumen of the gut (figure

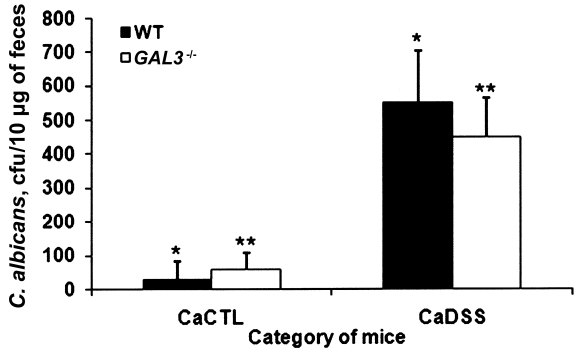

Figure 1. *Candida albicans* cfu recovered from stools of wild-type mice (WT; *black bars*) and *Gal3*$^{-/-}$ mice *(white bars).* Each data set represents the mean value of *C. albicans* counts for CaCTL mice (control group of mice inoculated with *C. albicans* by oral gavage) and CaDSS mice (mice inoculated with *C. albicans* by oral gavage and treated with DSS for 7 days). * and **, significant differences ($P < .05$) between corresponding groups.

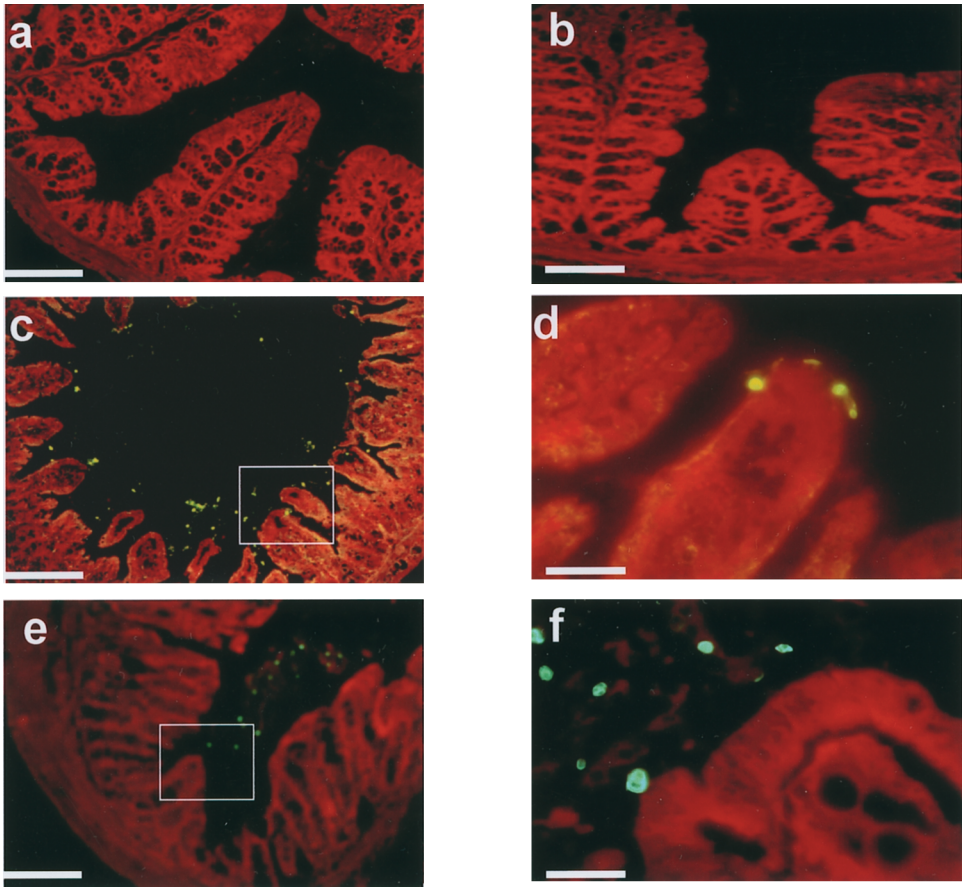

Figure 2. Immunofluorescence staining of colon sections with an anti–β-1,2–linked oligomannose monoclonal antibody specific for *Candida albicans*. Representative sections of colon from wild-type mice (WT; *panel a*) and *Gal3*$^{-/-}$ mice *(panel b)* inoculated with *C. albicans* alone. No yeast can be observed. *Panel c* and *e,* WT mice *(c)* and *Gal3*$^{-/-}$ mice *(e)* treated with DSS and inoculated orally with *C. albicans. C. albicans* blastoconidia can be observed in the colon lumen and adhering to epithelial cells. Boxed regions in *panel c* and *e* are shown at a higher magnification than in *panel d* and *f.* The scale bars represent 50 μm *(panels a, b, c* and *e)* and 10 μm *(panels d* and *f).*

2*a*) or adhering to the epithelium (figure 2*b*). We did not find any hyphae or pseudohyphae, nor did we observe epithelial invasion in any of the large number of sections examined.

Detection of anti-yeast mannan antibodies in serum from mice. We investigated the effect of DSS administration on the serologic response to yeast mannans. In both WT and *Gal3*$^{-/-}$ mice, oral administration of *C. albicans* to mice receiving DSS resulted in a significant production of anti–*C. albicans* mannan antibodies (figure 3*A*). Because *C. albicans* infection can induce an ASCA response, we also investigated the effect of *C. albicans* colonization and DSS treatment on the production of ASCA. As shown in figure 3*B*, mice that received DSS and *C. albicans* produced ASCA. With almost similar backgrounds for both tests, even higher increases in absorbance were detected in the ASCA ELISA as opposed to the *C. albicans*–specific mannan ELISA. The receipt of *C. albicans* alone did not lead to the generation of these antibodies (data not shown).

Determination of histologic scores and MPO activity. Histologic analysis showed a significant difference in the number of colon lesions between DSS-treated WT mice and DSS-treated WT mice that received *C. albicans* by gavage ($P < .05$; figure 4*A* and figure 4*B*). By contrast, in *Gal3*$^{-/-}$ mice, DSS-induced histologic scores did not significantly increase after *C. albicans* gavage (figure 4*A* and figure 4*B*). As one of the hallmarks of induced colitis is marked infiltration of neutrophils into the mucosa, this was estimated by measuring mucosal MPO activity. DSS administration increased MPO levels in the colon of WT mice compared to *Gal3*$^{-/-}$ mice ($P < .05$). Colonization with *C. albicans* still enhanced MPO activity in the colon of WT mice with DSS-induced colitis, an augmentation which was less marked in *Gal3*$^{-/-}$ mice treated with DSS and *Candida* ($P < .05$; figure 4*C*). Thus, MPO activity paralleled colonic damage.

Inflammatory cytokine and TLR mRNA expression in* Gal3$^{-/-}$ *mice and WT mice. To assess the specific contribution of proinflammatory cytokines and pathogen recognition receptors to the observed tissue damage, reverse transcriptase polymerase chain reaction (RT-PCR) amplification of mRNA isolated from colonic tissue was carried out for NOD-2, TLR-4, TLR-2, TNF-α and IL-1β (figure 5). DSS treatment and *Candida*

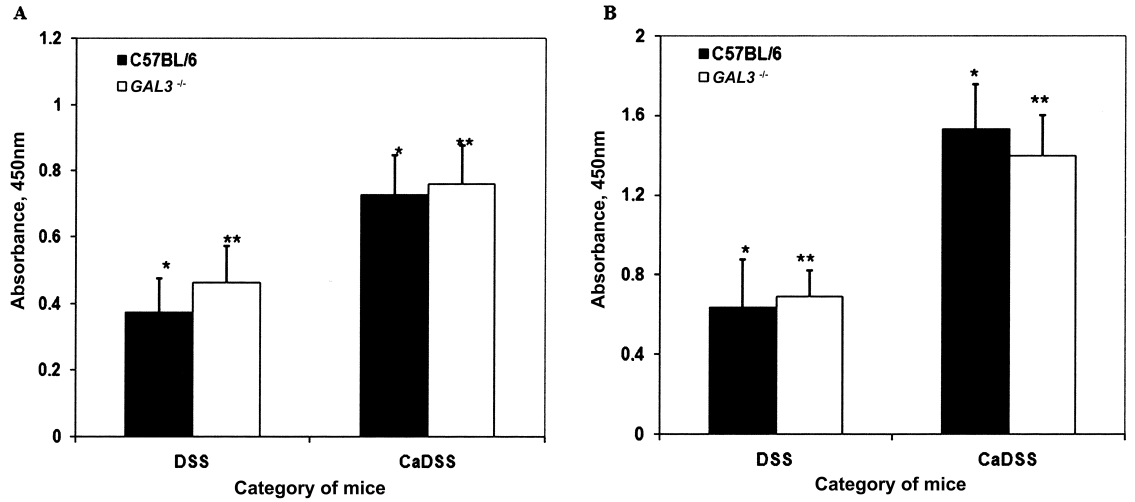

Figure 3. Detection of anti-yeast mannan antibodies in serum from DSS-treated mice with or without *Candida albicans* administration. Results are expressed as the mean of optical densities (± SE) observed in serum from each group of mice: wild type (WT; *black bars*) and *Gal3*$^{-/-}$ *(white bars),* compared with *C. albicans* mannan *(A)* and anti–*Saccharomyces cerevisiae* mannan antibodies (ASCA) *(B).* * and **, significant differences ($P < .05$) between the corresponding groups.

colonization, or both, had little effect on NOD-2 and TLR-4 expression. Expression of these pathogen recognition receptors was not increased by the presence of *C. albicans* in DSS-treated mice. DSS alone induced TLR-2 mRNA expression that was less marked in *Gal3*$^{-/-}$ mice, but TLR-2 mRNA levels were clearly enhanced by *C. albicans* colonization and treatment with DSS alone in WT mice. TNF-α expression paralleled TLR-2 mRNA production. It was increased in WT colonized mice, whereas no change was observed in *Gal3*$^{-/-}$ mice. In contrast, for IL-1β levels, which responded significantly to DSS in WT and *Gal3*$^{-/-}$ mice (although at a lower level for *Gal3*$^{-/-}$ mice), *C. albicans* colonization enhanced the response in both types of mice.

DISCUSSION

Several models have been developed to render the mouse, recognized as the most convenient laboratory animal, susceptible to colonization by *C. albicans.* Numerous papers have been published with results based on the use of infant mice [7] or mice treated with antibiotics [8–10]. In this study, we have established a new and simple model of murine colonization by *C. albicans* that involves DSS-induced colitis. DSS is thought to induce mucosal injury and inflammation, initially through a direct toxic effect on epithelial cells, with subsequent recruitment and activation of inflammatory cells and upregulation of inflammatory mediators, leading to the development of severe colitis [23]. This model was chosen over a variety of other chemicals used to induce colonic inflammation, such as 2,4,6-trinitrobenzenesulfonic acid [24, 25], acetic acid, phorbol ester, sulfated polysaccharides [26], and formalin [27], which are limited by the lack of chronicity and rapid colonic healing. This DSS treatment, which is usually proposed for mimicking several pathologic conditions of humans, resulted in a significant increase in the number of *C. albicans* colonies recovered from murine stool samples. Examination by immunofluorescence suggested that isolation of colonies reflected the presence of *C. albicans* in the gut, because large numbers of yeasts were found, either in the lumen of the colon or adhering to epithelial cells.

Gal-3 is an endogenous lectin, involved in a variety of normal and pathologic processes [28, 29]. Gal-3 also has an important role in inflammation [30]. In inflammatory bowel diseases, Gal-3 has been shown to be an autoantigenic target following cleavage after damage of epithelial cells [31] or impairment of its proapoptotic role [32]. Regarding *C. albicans,* Gal-3 acts as a receptor for β-mannose adhesins expressed at the cell wall surface [12, 13]. In the present study, we used *Gal3*$^{-/-}$ mice to investigate the effect of Gal-3 on *C. albicans* colonization and its relationship with inflammation.

After administration of DSS, *Gal3*$^{-/-}$ mice displayed lower histologic scores for inflammation. This was confirmed by determination of MPO activity, as a parameter of neutrophil accumulation, which was higher in the colon of WT mice with DSS-induced colitis, compared with *Gal3*$^{-/-}$ mice. Similarly, for pathogen recognition receptors and cytokine genes whose transcription was stimulated by DSS (i.e., TLR-2, IL1-β, and TNF-α), their induction was reduced significantly in *Gal3*$^{-/-}$ mice. All of these results strongly support a role for Gal-3 in inflammation.

When considering *C. albicans*–host interactions against this DSS background, the first observation was that the absence of *Gal3* did not significantly reduce the level of colonization by *C. albicans,* compared with WT mice. This was a surprise in view of the strong expression of β-mannose epitopes at the *C. albicans*

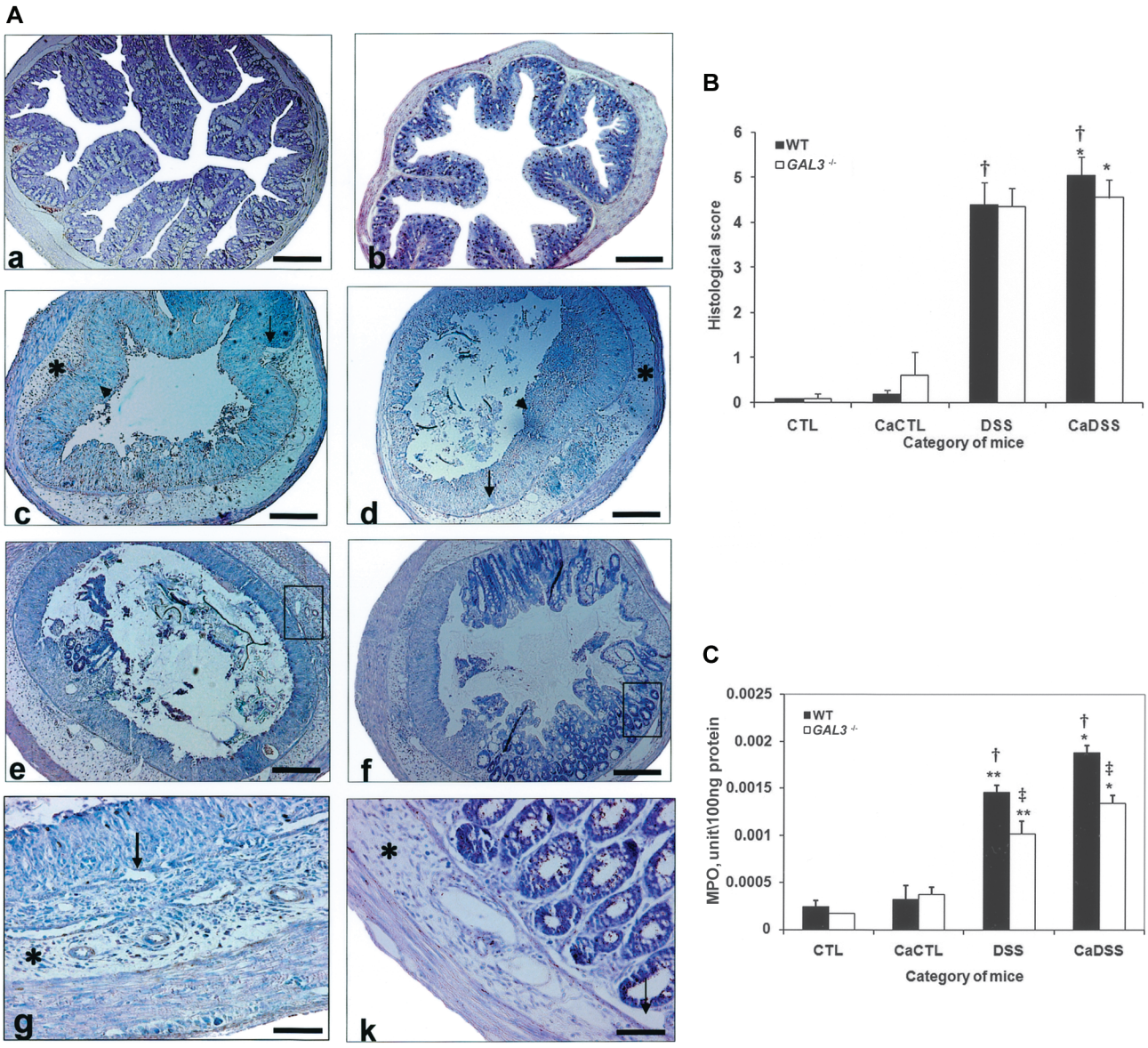

Figure 4. Histology and assessment of infiltrating neutrophil activity. *A,* Histologic characteristics of colon sections from wild-type (WT) and *Gal3*$^{-/-}$ mice. Sections (4 μm thick) stained with May-Grünwald-Giemsa stain. At low magnification *(panels a–f),* living cells (e.g., *panel a* and *b*) display a typical purple color, whereas the gray color (e.g., *panel c* and *f*) indicates direct cell damage and death. *Panel a* and *b,* control colon sections from WT *(a)* and *Gal3*$^{-/-}$ *(b)* mice. *Panel c* and *d,* colon sections from WT *(c)* and *Gal3*$^{-/-}$ *(d)* mice with dextran sulfate sodium (DSS)-induced colitis. *Panel e* and *f,* colon sections from mice with DSS-induced colitis inoculated with *C. albicans* by oral gavage; WT *(e)* and *Gal3*$^{-/-}$ *(f)* mice. Portions of boxed regions in *panel e* and *f* are shown at a higher magnification than in panels *g* and *k.* Compared with colon sections from control animals *(a* and *b),* the colon sections from DSS-treated mice *(c* and *d)* showed severe inflammation with large numbers of infiltrating cells *(asterisk),* tissue destruction with almost complete loss of crypts, loss of epithelial integrity *(arrowheads),* and edema *(arrows).* In the presence of DSS, *Candida albicans* gavage generated the same morphologic damage in both WT and *Gal3*$^{-/-}$ mice with an extensive cellular infiltrate especially neutrophil cells (*asterisk,* panels g and k), submucosal edema (*arrows,* panels g and k), and epithelial destruction (*arrowhead,* panel g), but cellular inflammation was more pronounced in WT mice *(e).* The scale bars represent 250 μm *(panels a, b, c, d, e,* and *f)* and 25 μm *(panel g* and *k). B,* Histologic scoring performed by 2 independent, blinded examiners (196 examinations). Data are expressed as mean ± SE for each group. WT and *Gal3*$^{-/-}$ mice (*black bars* and *white bars,* respectively); CaCTL mice (control group of mice inoculated with *C. albicans* by oral gavage); and CaDSS mice (mice inoculated with *C. albicans* by oral gavage and treated with DSS for 7 days). *, **, and † , significant differences ($P < .05$y) between the corresponding groups. *C,* Myeloperoxidase (MPO) activity. Data are expressed as mean ± SE for each group. †, $P < .05$ for CaDSS WT vs. DSS WT mice; ‡, $P < .05$ for CaDSS *Gal3*$^{-/-}$ vs. DSS *Gal3*$^{-/-}$ mice; *, $P < .05$ for CaDSS WT vs. CaDSS *Gal3*$^{-/-}$ mice; and **, $P < .05$ for DSS WT vs. DSS *Gal3*$^{-/-}$mice.

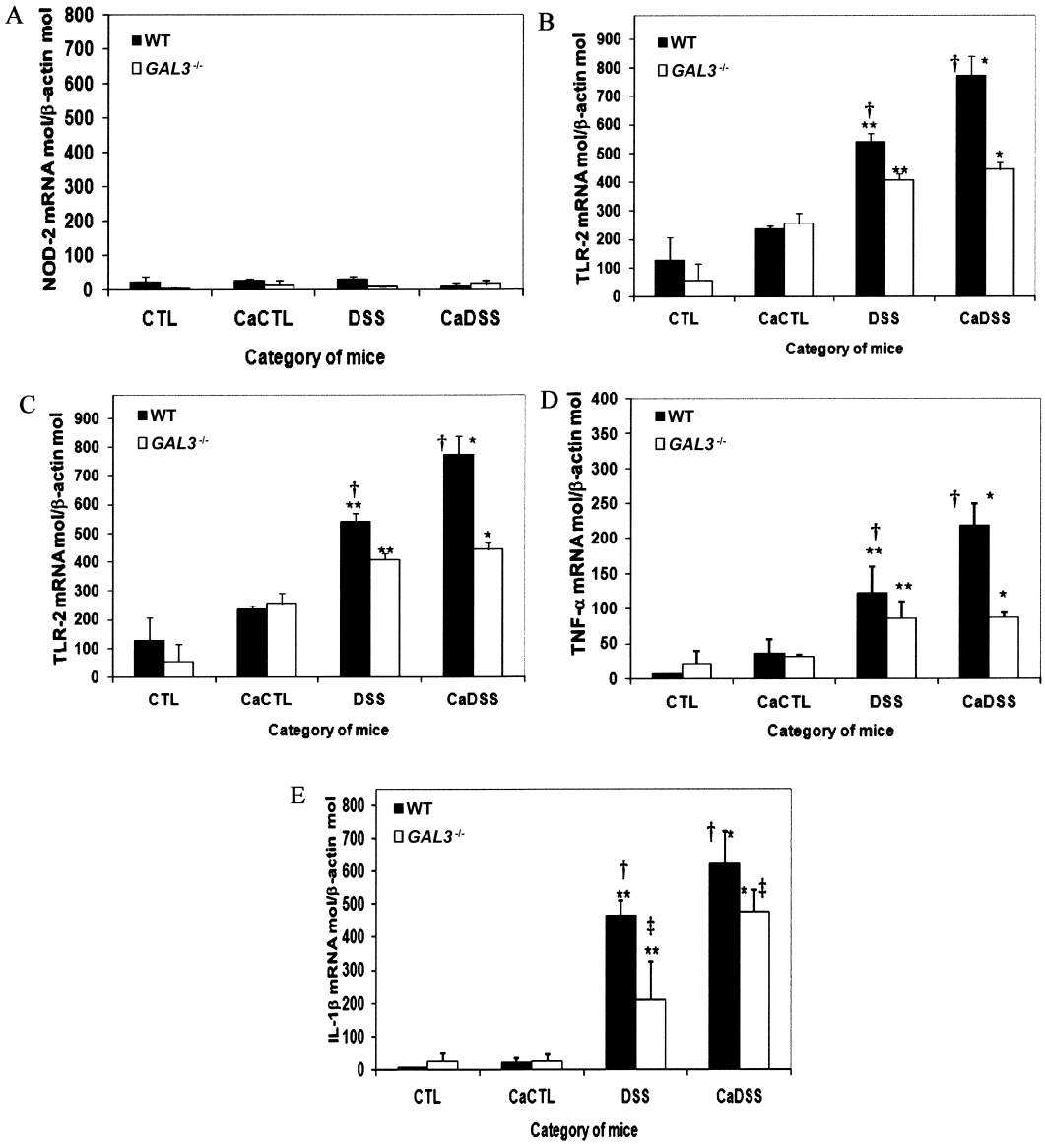

Figure 5. Relative expression levels of NOD-2 *(A),* TLR-4 *(B),* TLR-2 *(C),* TNF-α *(D),* and IL-1β *(E)* mRNA in mouse colons. Data are expressed as the means ± SE for each group. Wild-type (WT) and *Gal3*$^{-/-}$ mice (*black bars* and *white bars,* respectively); CaCTL mice (control group of mice inoculated with *Candida albicans* by oral gavage); CaDSS mice (group of mice inoculated with *C. albicans* by oral gavage and treated with DSS for 7 days). †, ‡, *, and **, significant ($P < .05$) differences between the corresponding groups.

cell-wall surface (figure 2), which are ligands for Gal-3. However, this reflects the multiplicity of *C. albicans* adhesins in epithelial cells [4, 33].

Among the different possible interactions involved in *C. albicans* epithelial cell adherance, most molecular information has been gained over the past few years on glycan–pathogen recognition receptor interactions. The pathogen recognition receptors involved in *C. albicans* recognition comprise TLR -2, TLR-4 and TLR-6—among which TLR-2 and TLR-4 have prominent roles [34]—and endogenous lectins, including mannose-mannan–binding and glucan-binding lectins. Mannose-mannan–binding lectins are distributed among C-lectins specific for α-linked mannose residues, such as mannose-binding lectin [35], the mannose receptor present on macrophages [36], DC-SIGN (dendritic cell-specific intracellular adhesion molecule [ICAM]-grabbing nonintegrin) [37], Dectin-2 [38], and an S-lectin, galectin-3, that binds β-linked mannoses specific for *C. albicans* (the topic of the present study) [12, 39]. Finally, Dectin-1 recognizes *C. albicans* β-glucans [40].

The absence of *C. albicans* colonization in *Gal3*$^{-/-}$ mice is in agreement with previous in vitro observations on the role of this lectin in the binding and endocytosis capabilities of macrophages from *Gal3*$^{-/-}$ mice and leads to the conclusion that Gal-3 is not critical for binding/engulfment of *C. albicans* [14]. Numerous studies in humans have emphasized the role of *C. albicans* colonization in the generation of circulating anti-mannan

antibodies [41, 42]. Treatment with DSS alone did not lead to the generation of such antibodies in mice. However, in this model, we confirmed the generation of anti–*C. albicans* mannan antibodies when *C. albicans* colonization was established. In this set of experiments, Gal-3 status had no influence on anti–*C. albicans* antibody levels. Interestingly, when these mouse serum samples were allowed to react with a *S. cerevisiae* mannan antigen, a significant antibody response was also observed. Anti–*S. cerevisiae* mannan antibodies are known as ASCA. ASCA are used extensively as serologic markers of Crohn disease, a chronic inflammatory bowel disease the etiology of which is still unknown but is considered to be a genetically based lack of tolerance to microbial and/or luminal antigens. It was shown that the major oligomannose epitopes of *S. cerevisiae* mannans supporting the ASCA response were expressed by the pathogenic phase of *C. albicans.* This study suggested that *C. albicans* could be an immunogen for ASCA [3]. The data obtained with the present model show that DSS damage alone did not permit ASCA generation by endogenous fungal microbiota. This result is in agreement with previous observations showing that the administration of *S. cerevisiae* extracts to DSS-treated mice failed to generate ASCA [43]. Morever, our results confirm that when *C. albicans* encounters an inflamed bowel microenvironment, it triggers ASCA production.

An unexpected observation was that *C. albicans* augmented the inflammation induced by DSS. This was clearly demonstrated in WT mice by histologic scores and MPO activity, as well as IL1-β and TNF-α expression. RT-PCR results for differential expression of pathogen recognition receptors also showed that TLR-2 expression was increased by *C. albicans.* In contrast, TLR-4 mRNA expression was not induced by *C. albicans* stimulation. We also investigated NOD-2 because this sensor of endogenous microbes participates in genetic susceptibility to inflammatory bowel diseases [44]. Our data ruled out a role for NOD-2 in sensing *C. albicans.* This is in accordance with a recently published study that demonstrated that NOD-2 was not involved in the recognition of *C. albicans* [45].

The last objective of this study was to define a role for Gal-3 in the sensing of *C. albicans* by comparing augmentation of the inflammation induced by *C. albicans* in WT and *Gal3*$^{-/-}$ mice treated with DSS. First, the absence of *Gal3* reduced histologic scores. Qualitative analysis revealed that, for MPO activity and IL-1β levels, although the baseline values were lower than those observed for WT mice, increases resulted from *C. albicans* stimulation. In contrast, RT-PCR analysis revealed that TLR-2 and TNF-α expression, which were amplified by *C. albicans* stimulation in WT mice, became completely unresponsive to this stimulus in *Gal3*$^{-/-}$ mice.

It is now established that the control of pathogens is based on proinflammatory and anti-inflammatory responses, an excess of which could be deleterious to the host by means of immunopathogenic mechanisms or by direct microbial damage, respectively [46]. This applies to *C. albicans,* which is tolerated in the gut [47], can invade it [48, 49], and can cause inflammation [50, 51].

This experimental study has identified a role for *C. albicans* in the augmentation of intestinal inflammation for the first time, to our knowledge. It also showed that *C. albicans* induced the formation of ASCA in the intestinal niche [3]. These results support previous data concerning the link between Gal-3 and intestinal inflammation, as well as the growing body of evidence concerning the involvement of Gal-3 in *C. albicans*–host interactions [13]. A major role for TLR-2 in the recognition of *C. albicans* at the intestinal level was highlighted, in agreement with the results of a previous in vitro study that demonstrated that TLR-2 and Gal-3 associate to stimulate TNF-α synthesis [14]. This in vivo model shows that in the absence of Gal-3, neither TLR-2 nor TNF-α synthesis were stimulated by *C. albicans.* Beside these specific conclusions, this new and easy model of mouse colonization may be valuable for further studies on molecular mechanisms controlling host–*C. albicans* interactions in the intestinal niche.

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