Saccharomyces cerevisiae CNCM I-3856 Prevents Colitis Induced by AIEC Bacteria in the Transgenic Mouse Model Mimicking Crohn’s Disease

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Background: Adherent-invasive Escherichia coli (AIEC), which colonize the ileal mucosa of patients with Crohn’s disease (CD), are able to adhere to and invade intestinal epithelial cells. Overexpression of the glycoprotein CEACAM6 on host cells favors AIEC attachment and inflammation. We investigated the ability of Saccharomyces cerevisiae CNCM I-3856 to inhibit AIEC adhesion and to reduce colitis.

Methods: Adhesion experiments were performed on T84 cells and on enterocytes from patients with CD with AIEC LF82 in the presence of or S. cerevisiae and an increased abundance of members of the Faecalibacterium prausnitzii–Ruminococcus torques groups. Proinflammatory cytokines were quantified by enzyme linked immunosorbent assay. Intestinal permeability was assessed by measuring the 4 kDa dextran-FITC flux in the serum.

Results: S. cerevisiae strongly inhibited LF82 adhesion to T84 cells and to the brush border of CD enterocytes. Yeasts decreased LF82 colonization in CEABAC10 mice and restored barrier function through prevention of the LF82-induced expression of pore-forming tight junction claudin-2 at the plasma membrane of intestinal epithelial cells. These effects were accompanied by a decrease in proinflammatory cytokines IL-6, IL-1β, and KC release by the gut mucosa. Yeast derivatives exerted similar effects on LF82 colonization and colitis demonstrating that yeast viability was not essential to exert beneficial effects.

Conclusions: S. cerevisiae yeasts reduce colitis induced by AIEC bacteria in CEACAM6-expressing mice. Such a probiotic strategy could be envisaged in a subgroup of patients with CD abnormally expressing CEACAM6 at the ileal mucosa and therefore susceptible to being colonized by AIEC bacteria.

Key Words: Crohn’s disease, adherent-invasive Escherichia coli, probiotic, Saccharomyces cerevisiae, yeast cell wall
located on the top of the type 1 pili of AIEC bacteria, which interacts with mannose residues exposed on the surface of the glycoprotein CEACAM6 (carcinoembryonic antigen-related cell adhesion molecule 6), overexpressed at the ileal mucosa of patients with CD.\textsuperscript{18} AIEC reference strain LF82 is able to persist in the gut of yeast strain Saccharomyces cerevisiae var. boulardii,\textsuperscript{21,22} mimicking the interaction between AIEC bacteria and host observed in CD. In this mouse model, AIEC colonization led to increased intestinal permeability and severe colitis.\textsuperscript{19,20}

Given that IBD can result from the presence of harmful bacteria, therapeutic strategies based on the use of antibiotics or probiotics could be a viable option. In both CD and UC, antibiotics have been used with some efficacy, but in certain studies, they failed to obtain or maintain remission (for reviews, see Refs. 21, 22). Probiotics represent an alternative strategy for preserving the delicate balance of intestinal microbiota in patients with IBD. Meta-analyses of clinical trials evaluating the effectiveness of probiotics in patients with CD failed to demonstrate any significant benefit.\textsuperscript{23,24} Only 2 clinical trials, one using \textit{E. coli} Nissle 1917 and the other \textit{Saccharomyces cerevisiae var. boulardii}, have yielded positive results of probiotics in patients with CD, in both cases by extending the remission period of the disease.\textsuperscript{25,26} CD is a disease with various etiologies and so, patients should be stratified before treatment. Failure to do so could explain why some probiotic therapies have been unsuccessful.

The present study aims to investigate the possible use of a potential probiotic yeast strain as an alternative treatment of ileal CD in which AIEC are involved in the chronic inflammatory process. We assessed the ability of the \textit{S. cerevisiae} CNCM I-3856 strain to capture AIEC bacteria through the high content of mannose residues on the yeast cell wall that can be recognized by type 1 pili expressed on the bacterial surface. We hypothesized that such an interaction could prevent AIEC colonization in susceptible hosts abnormally expressing mannansylated CEACAM6 at the ileal mucosa. The effect of yeasts was analyzed in vitro using cultured intestinal epithelial cells, ex vivo with ileal enterocytes isolated from patients with CD and in vivo in a transgenic mouse model expressing CEACAM6.

**MATERIALS AND METHODS**

**Bacterial and Yeast Strains**

The ampicillin–erythromycin resistant AIEC strain LF82 isolated from an ileal biopsy of a patient with CD was used as AIEC reference strain.\textsuperscript{16} The previously generated LF82-Δmfl isogenic mutant\textsuperscript{18} does not synthetize type 1 pili. The AIEC strains LF9, LF15, LF16, LF31, LF50, LF54, LF65, and LF110 had been previously isolated from the ileal mucosa of patients with CD.\textsuperscript{19} Bacteria were routinely grown at 37°C in Luria Bertani (LB) broth or on LB agar overnight. \textit{S. cerevisiae} yeast strain CNCM I-3856 and partially purified yeast cell wall extracts were provided by Lesaffre International (Marcq-en-Baroeul, France). Partially purified \textit{S. cerevisiae} yeast cell wall extracts were obtained after yeast autolysis, the insoluble cell wall being separated from the soluble yeast extract by centrifugation and then spray dried.\textsuperscript{27,28} Yeast products were rehydrated in phosphate-buffered saline (PBS, pH 7.2) at room temperature.

**Adhesion and Invasion Assays on T84 Cells**

Human intestinal epithelial cell line T84 was obtained from the American Type Culture Collection and was maintained in an atmosphere containing 5% of CO\textsubscript{2} at 37°C in the culture medium recommended by American Type Culture Collection. Cells were seeded in 24-well tissue culture plates at 4 × 10\textsuperscript{4} cells per well. Monolayers were infected at a multiplicity of infection of 10 bacteria per cell for 3 hours. \textit{S. cerevisiae} CNCM I-3856 was tested at doses ranging from 1 × 10\textsuperscript{5} to 1 × 10\textsuperscript{7} yeasts/mL. For coinoculation experiments, yeasts and bacteria were simultaneously incubated with cells. For preincubation experiments, cells were pretreated with yeasts 1 hour before bacterial infection, performed in the presence of the yeasts. For the postincubation protocol, cells were incubated with yeasts for 3 hours, after LF82 infection and washing steps to eliminate nonadherent bacteria. Numbers of cell-associated bacteria or intracellular bacteria were determined as previously described.\textsuperscript{29} Briefly, to assess bacterial adhesion after the 3-hour period of infection, monolayers were washed 3 times in PBS and then lysed with 1% Triton X-100 (Sigma) in deionized water. Samples were diluted and plated onto LB agar plates to determine the number of colony-forming units (CFU). To determine the number of intracellular bacteria, 100 μg/mL of gentamicin was added to the cell culture medium after infection for 1 hour to kill extracellular bacteria. Adhesion or invasion levels of AIEC bacteria in the absence of yeast were normalized as 100%, and the percentages of residual adhesion or invasion were expressed according to the yeast concentration.

**Adhesion Assays on Primary Ileal Enterocytes from Patients with CD**

Enterocytes were prepared from resection specimens obtained in a former study from 3 patients having undergone surgery for ileal involvement of CD.\textsuperscript{16} Isolated enterocytes were prepared from ileal specimens frozen at −80°C in MEM (Seromed Biochrom) containing 10% glycerol and 10% DMSO (Sigma) immediately after removal. Frozen intestinal samples were washed 3 times in PBS (pH 7.2), and the ileal mucosa was scraped with a coverslip to detach enterocytes. LF82 strain was previously demonstrated to strongly adhere to these isolated enterocytes through type 1 pili in a mannose-sensitive manner. Approximately, 10\textsuperscript{5} enterocytes were incubated for 15 minutes with \textit{S. cerevisiae} CNCM I-3856 at final concentrations of 2.5; 5 and 10 × 10\textsuperscript{7} yeasts/mL in DMEM supplemented with 20% of heat-inactivated fetal calf serum. AIEC LF82 bacteria were added at a concentration of 10\textsuperscript{8} bacteria/mL for 2 hours at 37°C with gentle shaking. After 3 washes in PBS, bacterial adhesion was quantified by examination under phase contrast microscopy at a magnification of ×1000. Only enterocytes with intact brush border were taken into account for the adhesion quantification. The number of \textit{E. coli} bacteria adhering to the brush border of 30 to 50 enterocytes was counted in duplicate. Experiments
with enterocytes were performed in duplicate by at least 2 different experimenters. The adhesion index of the LF82-ΔymH mutant was assessed and used as control.

### Mouse Infection Experiments and Ethics Statement

All mice were housed in specific pathogen-free conditions in the animal care facility at the University of Auvergne (Clermont-Ferrand, France). FVB/N (Friend Virus B NIH Jackson) WT mice were purchased from Charles River Laboratories, and CEABAC10 transgenic mice\(^{30}\) (homozygote) were maintained in our animal facilities. The animal protocols used in this study were approved by the CEMEA ("Comité d’Éthique en Matière d’Expérimentation Animale") Auvergne committee for ethical issues (permit CEMEAA CE16-09).

The infection protocol of CEABAC10 mice with AIEC LF82 bacteria for assessment of colonization and signs of colitis was performed as previously described, with minor modifications. Briefly, 8- to 10-week-old FVB/N CEABAC10 transgenic mice were given dextran sulfate sodium (molecular mass, 36,000–50,000 Da, MP Biomedicals) at 0.25% in drinking water, corresponding to a very low dose to increase the accessibility of bacteria to the surface of the epithelial layer, starting 3 days before infection. A group of mice was treated daily with 10⁸ yeasts (S. cerevisiae CNCM I-3856) or with 5 mg of yeast cell wall derivatives from S. cerevisiae by intragastric administration. Twenty-four hours after treatment with 5 mg of broad-spectrum antibiotic streptomycin (Euromedex) to disrupt normal resident bacteria to the surface of the epithelial layer, starting 3 days before infection. Yeast treatment started 10 days before infection at a dose of 10⁷ S. cerevisiae yeasts or 5 mg of S. cerevisiae cell wall extracts per mouse per day. Mice were infected twice at 14 days interval with 10⁶ CFU of the AIEC LF82 strain. Mice were orally challenged with 15 mg of FD4 diluted in PBS, 5 hours before blood collection. Serum was collected by centrifugation (30 min, 5000g) and FITC concentration was determined by fluorescence measurement and compared with a standard curve of FD4 diluted in serum. FITC was measured before infection to determine the basal level of intestinal permeability and 3 days after the second infection. Claudin-2 expression was assessed by immunofluorescence on a 0.5-cm sample of proximal colon of mice 3 days after the second infection. Snap-frozen colons in isopentane were embedded in optimum cutting temperature medium, stored at −80°C and cut into 8-μm slices with a cryostat. Samples were fixed in 1% paraformaldehyde for 20 minutes, washed in PBS. Permeabilization was performed with 0.5% Triton X-100 in PBS for 20 minutes. Unspecific sites were blocked using PBS/5% fetal bovine serum and 2% bovine serum albumin for 1 hour. Rabbit anti-claudin-2 (Abcam) antibody was diluted in blocking buffer (1/150) and incubated overnight at 4°C. After PBS washes, tissues were incubated for 90 minutes with a donkey anti-rabbit Cy3-conjugated secondary antibody diluted in PBS-fetal bovine serum 5% supplemented with Hoechst (1/500). Slides were mounted using Mountex-mounting medium (CellPath). Tissues were visualized using a confocal microscope Leica TCS SPE (Leica, Wetzlar, Germany).

### Statistical Analysis

Data are expressed as mean ± SEM or as medians. Data were compared using Student’s t-test analysis or nonparametric one-way analysis of variance Mann–Whitney test when appropriate. Differences were significant when \( P \) value was <0.05. Statistical analyses were performed using GraphPad Prism 5.00 (GraphPad Software, San Diego, CA) software package for PC.
RESULTS

*S. cerevisiae* Inhibits AIEC Adhesion to and Invasion of Intestinal Epithelial T84 cells

Effect of *S. cerevisiae* yeast strain CNCM I-3856 was investigated in vitro, in AIEC LF82 adhesion and invasion assays to intestinal epithelial cells T84. In the preincubation protocol, T84 cells were incubated with increasing concentrations of yeasts 1 hour before bacterial infection, leading to a strong and dose-dependent decrease in the number of cell-associated bacteria and the number of invasive bacteria (Figs. 1A and C). Incubation of cells with $5 \times 10^6$ and $1 \times 10^7$ yeasts/mL decreased the AIEC LF82 residual adhesion to 25.9% ± 2.8% and 11.8% ± 3.9%, respectively. In the coincubation experiment, the yeasts also exhibited a strong and dose-dependent inhibitory effect on the ability of the AIEC LF82 to adhere to and invade T84 cells (Figs. 1B and D). A significant inhibition of the LF82 adhesion was observed in the presence of $5 \times 10^5$ yeasts/mL of *S. cerevisiae*. Inhibitory effects on AIEC LF82 invasion paralleled those observed with the preincubation protocol, with a residual invasion level of 42.7% ± 10.6% at a dose of $5 \times 10^5$ yeasts/mL. At a maximal dose of $10^7$ yeasts/mL, residual invasion levels reached 6.7% ± 2.7% and 16.4% ± 6.7% for preincubation and coincubation assays, respectively. LF82 adhesion was finally assessed in

![Graphs of residual adhesion and invasion](https://academic.oup.com/ibdjournal/article/21/2/276/4602881)

**FIGURE 1.** *S. cerevisiae* reduces AIEC adhesion to and invasion of intestinal epithelial T84 cells. A–E, Adhesion and invasion assays: Infection of T84 cells with AIEC LF82 bacteria at a multiplicity of infection of 10 for 3 hours. A and C, Preincubation experiment: T84 cells were incubated with yeasts 1 hour before infection. B and D, Coincubation experiment: T84 cells were incubated simultaneously with yeasts and bacteria. E, Postincubation experiment: T84 were first infected with AIEC LF82 bacteria, cells were washed and incubated with yeasts for a 3-hour period. Results are expressed as percentages of adherent or intracellular bacteria, LF82 infection in absence of yeast was considered as 100% (mean ± SEM). *P < 0.05; **P < 0.01; ***P < 0.001.
incubating yeasts with cells after infection (postincubation). The yeasts exerted a moderate effect but significant enough to detach adherent bacteria from the cells with a residual adhesion of 60.1% ± 8.5% at doses up to $10^7$ yeasts/mL (Fig. 1E). The inhibition properties of $S$. cerevisiae were analyzed on 8 other AIEC strains isolated from ileal biopsies of patients with CD. In coinoculation experiments in the presence of $5 \times 10^6$ yeasts/mL, yeasts significantly decreased the levels of AIEC adhesion with residual adhesion levels ranging from 17.5% to 48.0% (Table 1).

**S. cerevisiae Prevents AIEC Adhesion to the Brush Border of Primary Ileal Enterocytes from Patients with CD**

We investigated the inhibitory effect of $S$. cerevisiae on the ability of AIEC LF82 bacteria to adhere to the brush border of enterocytes isolated from ileal biopsies of patients with CD selected for high CEACAM6 expression. In the absence of any treatment, LF82 adhesion indices to enterocyte brush border were 1.971, 1.921, and 0.933 bacteria per enterocyte for the 3 patients tested (Fig. 2). Treatment with yeasts significantly decreased the number of LF82 bacteria adhering to the brush border of enterocytes in a dose-dependent manner in all 3 patients with CD tested. A dose of $2.5 \times 10^7$ yeasts/mL was sufficient to significantly inhibit LF82 adhesion. At a concentration of $10^6$ yeast/mL, corresponding to a ratio of bacteria per yeast (1:1), AIEC LF82 adhesion indices were similar to those of the nonpiliated and nonadherent LF82-$\Delta fimH$ mutant, ranging from 0.019 to 0.177 bacteria per enterocyte. Thus, $S$. cerevisiae CNCM I-3856 was highly effective in preventing AIEC LF82 adhesion to enterocytes from patients with CD.

**S. cerevisiae Decreases AIEC LF82 Gut Colonization and Controls Disease Activity**

The ability of $S$. cerevisiae CNCM I-3856 to interfere with AIEC LF82 gut colonization was investigated in vivo in the CEABAC10 transgenic model expressing human CEACAM6. At day 5 postinfection, a significant 2-log decrease in the number of AIEC bacteria in the stools was observed in LF82-infected mice treated with $S$. cerevisiae compared with nontreated LF82-infected mice ($6.5 \times 10^3$ CFU/g and $2.8 \times 10^5$ CFU/g, respectively) (Fig. 3A). At day 7 postinfection, the amount of LF82 bacteria in mice receiving yeasts was significantly decreased compared with untreated mice. The effect of $S$. cerevisiae administration in the control of disease progression was assessed with the DAI score, on the basis of body weight loss, stool consistency, and the presence of blood in the feces. The body weight of AIEC LF82-infected mice was significantly lower from day 2 postinfection than that of noninfected animals (Fig. 3B). Interestingly, $S$. cerevisiae treatment significantly prevented loss of body weight from day 3 to day 7 postinfection ($P < 0.05$). The severity of colitis assessed at day 7 indicated that $S$. cerevisiae yeasts lowered the DAI score to a level similar to that of noninfected control mice (1.8 ± 0.6 and 1.9 ± 0.6, respectively; mean ± SEM) (Fig. 3C). Of note, 4 of 10 mice in the LF82 group presented blood in stools, whereas no blood was observed in the feces of the yeast-treated LF82-infected group. LF82 infection induced high morbidity with a survival rate of 30% at day 7 postinfection, whereas survival of AIEC-infected mice receiving yeasts was maintained at a rate of up to 70% (Fig. 3D).

Histological examinations by standard blinded histological scoring parameters of colonic tissues were performed to evaluate the degree of inflammation and colonic injuries. The histological score was significantly higher in LF82-infected mice that were not administered yeasts (5.6 ± 0.9) than in infected mice treated with

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**TABLE 1. Residual Adhesion of 8 AIEC Strains in a Preincubation Experiment to T84 Cells in the Presence of $5 \times 10^6$ Yeasts per milliliter of $S$. cerevisiae**

<table>
<thead>
<tr>
<th>AIEC strains</th>
<th>Mean ± SEM, (%)</th>
<th>t test</th>
</tr>
</thead>
<tbody>
<tr>
<td>LF9</td>
<td>28.87 ± 5.77</td>
<td>b</td>
</tr>
<tr>
<td>LF15</td>
<td>36.06 ± 2.06</td>
<td>b</td>
</tr>
<tr>
<td>LF16</td>
<td>30.80 ± 2.06</td>
<td>b</td>
</tr>
<tr>
<td>LF31</td>
<td>48.05 ± 17.16</td>
<td>a</td>
</tr>
<tr>
<td>LF50</td>
<td>35.56 ± 15.72</td>
<td>b</td>
</tr>
<tr>
<td>LF54</td>
<td>17.47 ± 3.52</td>
<td>c</td>
</tr>
<tr>
<td>LF65</td>
<td>20.43 ± 5.15</td>
<td>b</td>
</tr>
<tr>
<td>LF110</td>
<td>32.02 ± 12.20</td>
<td>b</td>
</tr>
</tbody>
</table>

a = $P < 0.05$; b = $P < 0.01$; c = $P < 0.001$ (t test) in comparison with adhesion in the absence of yeasts.

*Residual adhesion to the cells expressed in percentages (100% = adhesion in absence of yeasts).
yeasts (2.4 ± 0.5; P < 0.01), whose score was similar to that of the noninfected group (3.1 ± 0.7) (Fig. 4A). Massive neutrophil infiltration with transmural involvement, lymphoepithelial lesions, and even mucosal erosions were observed in the colonic mucosa of AIEC LF82-infected mice but not in those administered yeasts (Fig. 4B). A significant decrease in MPO activity in the colonic tissue was observed in response to yeast treatment in infected mice compared with that in LF82-infected but nontreated mice (P < 0.05), which, as in histological observations, was at a level similar to that of noninfected mice (Fig. 4C). Decreased levels of cytokines KC, IL6, and IL1-β were released by the colonic mucosa in LF82-infected mice treated with yeasts compared with nontreated LF82-infected mice (P < 0.05) at similar levels to those observed in noninfected mice (Figs. 4D–F).

Altogether, these data demonstrate that a daily administration of S. cerevisiae CNCM I-3856 favored the elimination of AIEC bacteria from the gut of CEACAM6-expressing mice and was able to control the colitis induced by AIEC LF82 colonization.

S. cerevisiae prevents barrier disruption induced by AIEC infection in CEABAC10 mice. As assessed in vivo by measuring FITC-labeled 4-kDa dextran (FD4) concentrations in the serum of LF82-infected CEABAC10 mice, administration of yeasts restored intestinal permeability to a basal level obtained before infection (Fig. 5A). It has been previously shown that the pore-forming tight junction protein Claudin-2 is overexpressed at the intestinal mucosa and associated with cell plasma membrane in response to AIEC infection.20 In AIEC-infected mice treated with yeasts, Claudin-2 was no longer associated with cell plasma membrane (Fig. 5B). Thus, S. cerevisiae yeasts were able to maintain barrier integrity in mice infected with AIEC and to prevent AIEC-induced injuries.
Effectiveness of *S. cerevisiae* Yeast Cell Wall Derivatives

*S. cerevisiae* cell wall derivatives were administered to CEABAC10 transgenic mice infected with LF82 bacteria to determine whether they could have similar protective properties to those of live *S. cerevisiae* yeasts. Oral administration of yeast derivatives to CEABAC10 mice challenged with AIEC LF82 bacteria led to a less severe body weight loss (mean of 90.2% for LF82-infected mice treated with yeast derivatives as against 83.9% for LF82-infected mice without treatment) (Fig. 6A). At day 3 postinfection, mice treated with yeast cell wall components had a significantly lower DAI score than LF82-infected mice without yeast treatment (Fig. 6B). In parallel with the improvement in colitis, the levels of bacteria in feces were significantly decreased by yeast treatment at day 3 postinfection (Fig. 6C). Although treatment with yeast derivatives led to less severe epithelial damages in LF82-infected CEABAC10 mice, the decrease of histological score at day 4 postinfection was not significant (Fig. 6D). We next assessed the impact of yeast cell wall treatment on the intestinal permeability of CEABAC10 mice infected with LF82. FITC concentration measured in the serum was maintained at a level of 106.8% ± 13.95% in LF82-infected mice receiving yeast cell wall, whereas fluorescence was increased by 4-fold in the nontreated LF82-infected mice (Fig. 6E).

Altogether, these results indicate that yeast cell wall components are able to decrease AIEC LF82 gut colonization and the symptoms of colitis and to protect intestinal mucosa against LF82-induced injuries.

FIGURE 4. *S. cerevisiae* decreases mucosal injuries induced by AIEC LF82 infection in transgenic mice expressing CEACAM6. A and B, Colonic tissues of LF82-infected CEABAC10 mice treated or not with 10⁸ yeasts per day of *S. cerevisiae* yeasts were collected at day 5 postinfection and stained by hematoxylin–eosin–safran to obtain a histological score (n = 8 per group) (mean ± SEM). C, Colonic MPO activity, expressed in units per gram of proteins (horizontal bars = medians) was measured in the colon at day 5 postinfection. D–F, Cytokine releases by proximal colon specimens at day 5 postinfection were measured by enzyme-linked immunosorbent assay (mean ± SEM). Noninfected control group = NI (n ≥ 6 per group). *P < 0.05; **P < 0.01.
DISCUSSION

Although the etiology of IBD is still unclear, the main hypothesis is that both UC and CD result from a dysregulated response of the intestinal immune system to antigens of microbial origin or pathogenic bacteria in genetically predisposed individuals. Culture-dependent and culture-independent analyses of mucosal-associated and fecal bacteria revealed that patients with IBD have less complex profiles of commensal bacteria and higher numbers of mucosa-associated bacteria than healthy individuals. Several independent studies have reported increased numbers of mucosa-associated \textit{E. coli} bacteria with invasive properties or the presence of intramucosal \textit{E. coli} in patients with IBD. \textit{E. coli} strains associated with the intestinal mucosa from patients with CD are highly adherent to intestinal epithelial cells and are also invasive\cite{11,16}. These CD-associated \textit{E. coli} strains, named AIEC for adherent-invasive \textit{E. coli}, were isolated from ileal specimens of 36.4% of patients with CD versus 6% of controls.\cite{17}

Manipulating enteric microbiota to reduce the number of “harmful bacteria” can be achieved with the use of antibiotics, probiotics, prebiotics, or combination therapies. Antibiotics have been used with some efficacy in CD, although routine use has been limited by the development of antibiotic resistance and systemic side effects.\cite{21,22,26} Probiotics represent another way of modulating gut microbiota, but to date clinical trials have failed to significantly demonstrate they have any beneficial effects in maintaining remission of CD. A small clinical trial involving 32 patients suggested a role for the yeast strain \textit{S. cerevisiae} var. \textit{boulardii} in preventing relapse in patients with CD in clinical remission\cite{25} but these results were not confirmed in a larger trial with 165 patients.\cite{38} Studies using murine models have shown that \textit{S. cerevisiae} yeast strains can prevent gastroenteritis due to \textit{Salmonella enterica} serovar Typhimurium.\cite{39,40} Direct binding of \textit{S. enterica} serovar Typhimurium onto the cell surface of \textit{Saccharomyces} yeasts has been observed in studies using transmission electronic microscopy or aggregation assays, probably because of their expression of type I fimbriae, which bind yeasts through recognition of mannan oligosaccharides of the yeast cell wall.\cite{41,42} It was interestingly demonstrated that \textit{S. enterica} serovar Typhimurium can bind to high-mannose type oligosaccharides of the family of CEACAM molecules on the intestinal epithelial cells through lectins on bacterial type I fimbriae.\cite{43} These experiments indicate that \textit{Saccharomyces} yeasts are able to capture enteric bacteria onto their cell surface, and that such a binding could prevent bacterial adhesion to their host receptors on the intestinal epithelium and subsequent invasion of the host mucosa.

Several \textit{S. cerevisiae} yeasts were reported to be able to trap \textit{E. coli}, \textit{S. enterica} serovar Typhi or \textit{S. enterica} serovar Typhimurium in vivo onto the yeast surface.\cite{44} AIEC bacteria adhere to intestinal epithelial cells mainly through type I pili, involving adhesin FimH, which binds to mannosic residues on glycoproteins expressed at the surface of host cells. With the identification of the major AIEC receptor CEACAM6, a highly glycosylated protein abnormally expressed at the ileal mucosa of 35% of patients with CD, it would be of value to investigate probiotic strategies. We previously showed that AIEC adhesion to enterocytes from

**FIGURE 5.** \textit{S. cerevisiae} prevents increase in intestinal permeability and the cell membrane localization of the pore-forming tight junction Claudin-2 induced by AIEC LF82 infection in transgenic mice expressing CEACAM6. A, FITC fluorescence was measured in the serum of AIEC LF82-infected CEABAC10 mice 5 hours after the intragastric administration of FITC-dextran 4 kDa (FD4). Results are expressed in micrograms of FD4 per milliliter of serum (mean ± SEM) (n = 5 per group). NT = nontreated mice. B, Claudin-2 expression was assessed by immunofluorescence (in red) on colonic cryosections of noninfected CEABAC10 mice, LF82-infected CEABAC10 mice, and LF82-infected CEABAC10 mice treated with 10⁷ \textit{S. cerevisiae}. DNA was stained blue using Hoechst.
patients with CD could be inhibited by specific antibodies directed against CEACAM6. Based on the fact that yeast cell wall is composed of high level of carbohydrates, we elected in this study to test the ability of \textit{S. cerevisiae} yeast strain CNCM I-3856 to prevent AIEC colonization by inhibiting FimH/CEACAM6 interaction. In vivo experiments in the transgenic mouse model CEABAC10-expressing human CEACAMs, a model particularly suitable for mimicking the AIEC gut colonization observed in patients with CD in reproducing the abnormal CEACAM6 overexpression in the intestinal mucosa, showed that administration of \textit{S. cerevisiae} yeasts decreased AIEC gut colonization. These results are in correlation with in vitro experiments because a strong inhibitory effect of \textit{S. cerevisiae} yeasts was observed on LF82 adhesion to intestinal epithelial cells and on LF82 adhesion to the brush border of enterocytes from ileal biopsies of patients with CD. In addition, we observed that administration of \textit{S. cerevisiae} to CEACAM6-expressing transgenic mice infected with AIEC bacteria was able to decrease the severity of colitis, the proinflammatory response, and mucosal injuries and to restore intestinal barrier function impaired by AIEC infection.

The ability of \textit{S. cerevisiae} var. \textit{boulardii} to control gastrointestinal infection has been previously documented with enteropathogenic \textit{E. coli} and enterohemorrhagic \textit{E. coli} (EHEC) pathovars. Yeasts prevented in vitro enteropathogenic \textit{E. coli}-induced and EHEC-induced decrease in transepithelial resistance and IL-8 secretion of infected intestinal epithelial cells. 

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure6.png}
\caption{\textit{S. cerevisiae} cell wall derivatives decrease AIEC LF82 gut colonization and symptoms of colitis in transgenic mice expressing CEACAM6. A–C, CEABAC10 mice were treated daily or not with 5 mg per mouse of yeast cell wall derivatives and infected with $10^9$ LF82 bacteria at day 0 (n = 10 per group). A, Evolution of body weight in percentages (mean ± SEM). B, Assessment of the DAI score at day 3 postinfection (mean ± SEM). C, Quantification of LF82 bacteria in the feces of LF82-infected mice (triangles) or LF82-infected mice receiving yeast cell wall derivatives (squares) at day 3 after infection. Horizontal bars represent medians. D, Assessment of the histological score of colonic tissues stained by hematoxylin–eosin–safran of LF82-infected CEABAC10 mice treated or not with 5 mg per mouse per day of yeast cell wall derivatives at day 4 postinfection (mean ± SEM). E, Assessment of the flux of FITC-dextran 4 kDa (FD4) in the serum of AIEC LF82-infected CEABAC10 mice, treated or not with yeast derivatives, 5 hours after intragastric administration of FD4. Results are expressed in percentages, concentrations were normalized by the FD4 basal levels measured before LF82 infection (mean ± SEM) (n = 5 per group). YD = yeast derivatives. *\textit{P} < 0.05.}
\end{figure}
However, unlike in this study, the presence of yeasts did not decrease the number of adherent bacteria. Of note, only live yeasts were observed to exert a protective effect against enteropathogenic *E. coli* and EHEC infection.

For safety reasons, we investigated whether yeast cell wall fractions can exert similar antiadhesive properties as whole yeasts because the use of live yeasts, which, while they are not pathogenic, could represent a risk for patients with CD with extensive damage of the intestinal mucosa. A few studies have reported that *S. cerevisiae*, normally considered as a nonpathogenic yeast, can be involved in fungemia in severely immunocompromized patients. Interestingly, we observed that yeast cell wall derivatives can decrease AIEC gut colonization and the DAI score of AIEC LF82-induced colitis in CEABAC10 mice as effectively as live yeasts.

The fact that no clinical trial with probiotic yeasts shows benefits in CD treatment points out the importance of yeast strain selection and the need for patient stratification. A primary step of screening of *S. cerevisiae* strains was performed to select the CNCM I-3856 strain for its high capacity to interact with AIEC bacteria in aggregation assays and to efficiently decrease AIEC LF82 adhesion to intestinal epithelial cells. Furthermore, this yeast strain led to a reduction of EHEC bacterial resumption in a dynamic gastrointestinal model. An antiadhesive therapy based on *S. cerevisiae* CNCM I-3856 has to be focused on patients with abnormal expression of CEACAM6 at the ileal mucosa and therefore susceptible to AIEC infection to decrease gut colonisation by AIEC and to control the subsequent inflammation. Administration of *S. cerevisiae* CNCM I-3856 yeast strain or yeast derivatives represent a promising strategy in ileal CD treatment and should be proposed to extend the remission period or to prevent postoperative recurrence.

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REFERENCES